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Short communication:

Induction of somatic embryogenesis in absence of exogenous auxin in cucumber (*Cucumis sativus* L.)

K. Mashayekhi, M. Sharifani, M. Shahsavand, H. Kalati

Horticulture Department, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
*Corresponding authors. E-mail:

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Abstract

Induction of somatic embryogenesis in absence of exogenous auxin can clarify some basic concepts of somatic embryogenesis. Explants from leaf and petiole of Cucumber *(Cucumis sativus)* were used in this experiment for somatic embryogenesis using three different modified B5 basal media; boron and 2,4-D but no IAA as control (I), boron and IAA but no 2,4-D (II) and no boron, 2,4-D and IAA (III). Leaf explants grown on III produced somatic embryos indicating an induction potential for somatic embryogenesis with an incomplete medium. The endogenous auxin of the leaf explants might have a potential for triggering induction of somatic embryogenesis.

Keywords: Cucumber; Embryogenesis; Tissue culture; Boron

Introduction

Cucumber *(Cucumis sativus)* as one of the important species of cucurbitacea family is cultivated extensively in many parts of the world. Lowering seed costs through somatic embryogenesis research could assist expansion of cucumber plantation. The main advantage of synthetic seeds is a mass propagation of plants which had desired traits in terms of yield, quality and resistance to biotic and abiotic stresses. Further using somatic embryogenesis allows a high multiplication rate followed by an established procedure in using suitable culture media and the handling of enormous number of embryos at one time (Merkle et al., 1995). Somatic embryo production depends mainly on such factors as genotype, explants type, age, and endogenous hormone level and stress conditions.

The exogenous auxin hormones raise the endogenous auxin level in the tissue (Neumann, 1995) increasing the occurrences of somatic embryogenesis.

A high level of IAA in boron deficient plants was reported by Marschner (2002). Decreasing free auxin level causes an increase in level of bound auxin and reduction of IAA-oxidase activity when boron is deficient (Cohen and Bandurski, 1978), increasing the

IAA level of the plant tissue (Paul et al., 1992; Cohen and Bandurski, 1978; Dugger, 1983). Bohnsack and Albert (1977) reported increased IAA oxidase activity in apical and sub apical root section of the plant when seedlings of squash were treated with both IAA and boron. Several physiological impairments are caused by boron deficiency such as inhibitions of mitosis, cell elongation, cell differentiation and development, respiration suppression, photosynthesis and an increased auxin content of the cells (Cohen and Bandurski, 1978). Boron deficiency reduced synthesis of uracil and hence mRNA and protein synthesis (Schilling, 2000). Boron affects the transport metabolism and activity of auxin (Robertson and Loughman, 1974). The ratio between cytokenin versus auxin is influenced by concentration of boron (Schilling, 2000; Mashayekhi and Neumann, 2006).

The objective of this experiment was to study the effect of boron deficiency on the callus initiation and somatic embryogenesis in a MS medium lacking auxin during the induction phase.

Materials and methods

This trial was carried out in tissue culture laboratory at Gorgan University of Agricultural Sciences and Natural Resources, Horticultural Department, Iran from April 6, 2004 to August 24, 2005. The first factor was explant as petiol and leaf lamina (two levels) and the second factor was modified B5 culture medium type with three levels. Surface sterilized ″Beth Alpha″ seeds were cultured in sterilized glass jars containing MS basal medium (Murashige and Skoog, 1962) solidified with 0.7% Agar for germination under constant temperature of 22ºC and 400 luxes of light using fluorescent light tubes. Leaf lamina and petiol of these in vitro-grown cucumber plants were used for somatic embryogenesis. Explants were harvested after 6-weeks. 5 pieces of 1 cm by 1 cm long leaf lamina and petiole explants were transferred to each vial containing a modified B5 medium (treatments 1 to 3). Each treatment for induction of somatic-embryogenesis had four replicates and each vial contained five explants. The pH of the modified B5 medium was adjusted to 5.7±0.1, autoclaved at 121ºC for 15 min, cooled to room temperature and dispensed into sterilized vials of 20 ml volume prior to transfer of explants. The explants cultures were incubated for 6 weeks at a constant temperature of 25ºC and 2000 luxes of light intensity using white fluorescent tubes. For the realization of the somatic embryogenesis, the explants from induction media were transferred to a B5 basal medium containing boron however lacking IAA and 2, 4-D. The plant materials in each vial were photographed using a stereomicroscope (Saairan, Iran).

Results and discussion

Leaf blades and petioles produced great amounts of callus with treatment I without initiation of embryogenesis. No adventives roots were observed with this treatment. Petioles and leaf lamina both produced little callus with treatment II. Adventitious roots were induced only in callus differentiated from leaf lamina by this treatment without initiation of embryogenesis (Figure 1). It could be as a result of a better response obtained from callus originated from the leaf lamina.

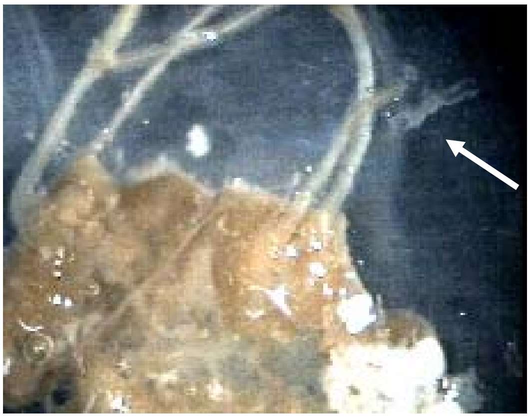

Figure 1. Induction of adventives roots in cucumber leaf tissue cultured in B5 basal medium containing boron and IAA however lacking 2, 4-D.

Despite lacking boron, 2, 4-D and IAA, treatment III induced embryogenesis with leaf blades, forming embryos with a globular structure. Microscopic observations showed somatic embryos originate from a large number of small cells with dense cytoplasm and small vacuoles in lower leaf area (Figure 2). Boron deficiency raises possibly the levels of endogenous auxin in callus initiating from the leaf lamina tissues. As a consequent, the vegetative cells could differentiate to generative ones. Petioles formed only callus but no embryos with treatment III. It appears the auxin concentration of petioles was only adequate for callus initiation and different plant organs have different potentials for embryo induction.

Figure 2. Induction of somatic embryos with leaf tissues using B5 medium in the absence of boron and exogenous auxin

Strolka (1996) reported an induction of embryogenesis in cucumber leaf explants with presence of 2, 4-D and 2-Ip (2-Isopentyl-adenine) using a MS medium. Other results demonstrate removal of 2, 4-D in realization phase could still lead to the emergence of embryos (Aly et al., 2002). Mashayekhi (2001) reported somatic embryogenesis in carrot tissue culture by a low boron during induction and realization phases possibly due to a reduced IAA oxidase activation in tissue and hence increased endogenous auxin. Dugger 1983, Cohen and Bandurski, (1978) reported non-peroxidative IAA oxidase might play a major role in the regulation of indole-3-acetic acid content in pea seedlings.

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