Differences in compounds released by embryogenic and non-embryogenic suspension cultures of Euphorbia pulcherrima

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Abstract

Media from embryogenic and non-embryogenic cell suspension cultures were analysed for protein content, electrophoretic protein patterns, glycoproteins and activity of peroxidases and β-glucosidases in order to characterize the physiological status of the cultures. On a dry mass basis the amount of extracellular proteins per cell was greater in embryogenic suspensions than in non-embryogenic suspensions. Non-embryogenic suspensions contained unidentified slimy compounds which were not present in embryogenic cultures. The extracellular Concanavalin A-specific glycoproteins gave different isoelectric focusing patterns and thus enabled embryogenic and non-embryogenic cultures to be differentiated. The extracellular peroxidase activity per cell dry mass was far greater in embryogenic than in non-embryogenic cultures. The isoenzymes differed in number and composition of the anionic bands. β-glucosidases were found in the same range of activity in both culture types, but the time course of enzyme activity during cultivation was significantly different. In the embryogenic culture the activity was correlated with dry mass increase, whereas in the non-embryogenic suspension the activity reached maximum during the linear growth phase. Polyphenoloxidase which was recently recognized as an intracellular marker for embryogenic stages was not released into culture media.

Additional key words: β-glucosidases, glycoproteins, peroxidases, somatic embryogenesis.

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Abbreviations: Con A - Concanavalin A; CPA - 4-chlorophenoxy-acetic acid; d.m. - dry mass; f.m. - fresh mass; IFP - isoelectric focussing; 5IP - 6-N-(2-isopentenyl)-adenine; MS - Murashige and Skoog; NAA - naphthaleneacetic acid; pI - isoelectric point.

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Introduction

In the course of differentiation processes changes in cell metabolism lead to significant changes in the culture medium. The exchange of high and low molecular mass components between the cells and the culture medium is of major importance for differentiation including somatic embryogenesis (De Vries et al. 1988, Wink 1984). The concentration of polyamines, especially putrescine and spermidine is greater in embryogenic than in non-embryogenic cell masses and the media of suspension cultures (Alman et al. 1983). Inhibition of polyamine synthesis reduces the number of embryos formed, and the addition of polyamines to inhibitor-containing cultures restarts embryo formation. The accumulation of ethylene, in contrast, is less in embryogenic suspension cultures than in non embryogenic cultures (Wann et al. 1989), as is the amount of glutathione. Differential uptake of carbon sources from the suspension media has been used as a biochemical marker for cultures that are embryogenic (Callebaut et al. 1987).

Extracellular compounds, especially glycoproteins and arabinogalactans found in suspension cultures have been analysed for their influence on developmental processes (e.g. Knob 1993: differentiation and morphogenesis; Sterk and de Vries 1993: embryogenesis; Hahn 1995: pathogenesis-induced processes). De Vries et al. (1988) proved that extracellular proteins and glycoproteins isolated from embryogenic carrot cell lines were able to partially restore somatic embryogenesis in cultures lacking one or several of those proteins. The significance of secreted proteins/glycoproteins in embryogenesis has been reviewed recently (Sterk and De Vries 1993, Hendriks and De Vries 1995). Kreuger and Van Holst (1993) showed extracellular arabinogalactans to be essential for somatic embryogenesis of carrots. Similar results were described for embryogenic suspensions of Picea abies (Arnold et al. 1985).

Specific changes in peroxidase isoenzymes relate to somatic embryogenesis of carrot (Joersbo et al. 1989); extracellular peroxidases released by embryogenic suspension cultures of carrot are important for the development of globular to heart-shaped carrot somatic embryos (Cordewener et al. 1991).

In the experiments described in this paper, extracellular substances in the medium of embryogenic and non embryogenic suspension cultures of Euphorbia pulcherrima were analysed in order to characterize different physiological states and to identify appropriate markers of somatic embryogenesis. The experiments focussed on the analysis of extracellular peroxidases, β-glucosidases, proteins and glycoproteins.

Materials and methods

Establishment of cell suspension cultures: Embryogenic cell suspension cultures of Euphorbia pulcherrima cv. Angelika were established according to Tricot and Beck (1991). Hypocotyl segments from somatic embryos were placed in Petri dishes on MS medium (Murashige and Skoog 1962) solidified with agar and supplemented with 0.2 mg dm$^{-3}$ naphthaleneacetic acid (NAA) and 0.1 mg dm$^{-3}$ N$\delta$-(2-isopropylamino)phenylacetic acid (IPA).