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Morphology, Histology and Protein Patterns in Embryogenic and Non-Embryogenic Callus of Carnation

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ABSTRACT

Somatic embryogenesis is a very useful system for studying basic biochemical, physiological and morphological aspects of plants. During somatic embryogenesis induction in carnation (*Dianthus caryopyllus* L.) cv. 'Nelson' two types of callus, embryogenic and non-embryogenic, were observed. The goal of this work was to compare the most relevant characteristics between these two callus types for a better understanding of the mechanism of somatic embryogenesis in carnation. Morphological and histological characteristics of both callus types were different. Morphohistological observations indicated a correlation between the morphological features of clusters and their embryogenic competence. On the other hand, protein patterns observed by SDS-PAGE showed differences that can explain that disparity. We propose that differential protein patterns can modulate the embryogenic capacity of carnation cells and that the number of proteins turned off in somatic cells to allows for the change from a somatic to an embryogenic state.

Keywords: *Dianthus caryopyllus*, embryogenesis
Abbreviations: 2,4-D, 2,4-Dichlorophenoxyacetic acid; **BA**, 6-benzylaminopurine; **MS**, Murashige and Skoog basal medium

INTRODUCTION

Carnation (*Dianthus caryopyllus* L.) is an important floricultural crop with high commercial interest worldwide (Burich *et al*. 1996). Somatic embryogenesis is a very valuable tool for achieving a wide range of objectives, from basic biochemical, physiological and morphological studies, to the development of technologies with a high degree of practical application (Zimmerman 1993; Vicient and Martínez *et al*. 1998).

Somatic embryogenesis is divided into two main phases: the first, whereby differentiated somatic cells acquire embryogenic competence and proliferate as embryogenic cells, and the second whereby the embryogenic cells display their embryogenic competence and differentiate into somatic embryos (Merkel *et al*. 1995). Embryogenic cells are unique: superficially they resemble meristematic cells, though they are generally smaller, more isodiametric in shape, have larger, more densely staining nuclei and nucleoli, and have a denser cytoplasm (Carman 1990; Feher *et al*. 2003).

Somatic embryogenesis has been reported for carnation (Frey *et al.* 1992; Sankhla *et al.* 1995; Yantcheva *et al.* 1998; Karami *et al*. 2006, 2008). However, even now, very little is known about the mechanisms of somatic embryogenesis induction in this ornamental plant. In this work we report some morphological, histological and protein patterns differences between embryogenic and non-embryogenic calli in carnation.

MATERIALS AND METHODS

Callus induction and histological examination

Embryogenic and non-ebryogenic calli were induced on MS medium containing 2 $mg l^{-1}$ 2,4-D, 0.2 $mg l^{-1}$ BA and 90 $g l^{-1}$ sucrose from petal explants of carnation as described by Karami *et al.* (2006). For histological investigation, both callus types were fixed in FAA (formalin: acetic acid: ethanol, 2:1:17, v/v) for 24 h, dehydrated in an alcohol series and then embedded in paraffin. Serial sections (7 µm) thick were cut with a microtome (Paya Pajohsh, Iran, 103-158) and stained with hematoxylin.

Protein extraction and electrophoresis

Embryogenic and non-embryogenic calli were frozen in liquid nitrogen and ground to a fine powder. Proteins were excreted in 100 mM Tris-HCl extraction buffer (pH 8.5) containing 4% (m/v) sodium dodecyl sulphate (SDS), 2% (v/v) β-mercaptoethanol, 20% (v/v) glycerol and 10 µg cm^{-3} leupeptin. The crude homogenate was boiled in water for 3 min and centrifuged at 14000 × *g* for 15 min. Electrophoresis was performed on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) 14% gels (SDS-polyacrylamide gel, Merck) according to Laemmli (1970) and the gels were stained with Coomassie brilliant blue. The molecular mass of protein was estimated by co-electrophoresis with marker proteins.

RESULTS AND DISCUSSION

During callus induction two types of callus could be recognized according to color, texture, and time of callus initiation. Type I calli was soft, succulent and yellowish green (**Fig. 1A**), and callus initiation started on the cut edges of explants within 2-3 weeks. Histological examination showed that cells of type I calli were longer and vacuolar and the relative sizes of nucleus smaller (**Fig. 1C**). Generally elongated and vacuolated cells are the non-embryogenic cells (Carman 1990; Feher *et al*. 2003). Therefore type I calli apparently are non-embryogenic calli. Type II calli were hard, creamy-white and nodular in texture (**Fig. 1B**) with a slow growing habit, and callus initiation was observed on the edges of the explants within 6-8 weeks. Histological examination showed that cells of type II calli had a dense cytoplasm, thick cell walls, and relatively bigger nucleus which resembled meristematic cells (**Fig. 1E**). Such characteristics of cells have already been observed in the embryogenic calli of *Allium ampeloprasum* (Buiteveld *et al*. 1994), *Gladiolus hort* (Stefaiank 1994) and *Agapanthus*

Received: 17 April, 2008. Accepted: 25 May, 2008.

Research Note

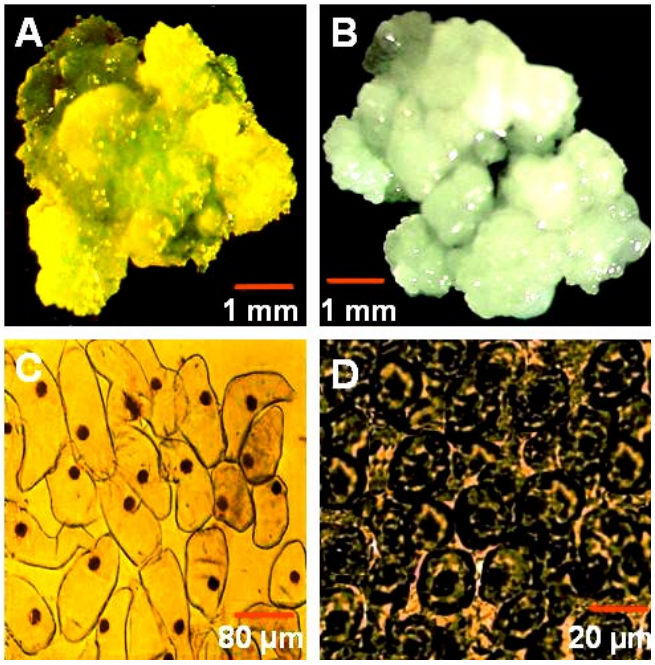

Fig. 1 Induction of embryogenic and non-embryogenic callus on MS medium containing 2 mg1^{-1} 2,4-D, 0.2 mg1^{-1} BA and 90 g1^{-1} sucrose in carnation cv. 'Nelson'. (**A**) Non-embryogenic callus. (**B**) Embryogenic callus. (**C**) Histological section of non-embryogenic callus. (**D**) Histological section of embryogenic callus.

praecox (Suzuki *et al*. 2002). Therefore type II calli are embryogenic calli that were apparently a mass of pro-embryogenic cells.

SDS-PAGE revealed different protein patterns of expression between embryogenic and non-embryogenic callus. In both calli, almost the same pattern was found (**Fig. 2**) except for minor qualitative differences. A similar result was reported between embryogenic and non-embryogenic callus in *Coffea arabica* (Quiroz-Figueroa *et al*. 2002).

Protein patterns revealed the existence of three proteins of 63, 20 and 7 kDa (arrows) that were specific in embryogenic callus and others of 72 and 31 kDa (arrowheads) specific to non-embryogenic callus (**Fig. 2**).

To induce somatic embryogenesis the existing gene expression pattern in the starting material must be modified and replaced with an embryogenic gene expression program in cells competent to give rise to somatic embryos (Hahne *et al*. 1988; Wurtele *et al.* 1993; Feher *et al.* 2003). The close similarity observed in the total protein patterns may be due to the fact that clusters have a common origin, but the presence of specific proteins in embryogenic callus and non-embryogenic callus could indicate that these may be involved in the determination process of embryo formation. These specific proteins could thus serve as biochemical markers for assessing the embryogenic capacity of *in vitro* cultures. Nevertheless, further biochemical studies will be necessary to understand the nature and role of these proteins.

In conclusion, and in brief, *in vitro* cultures having embryogenic callus and non-embryogenic callus from the same origin and cultured under the same conditions, offer the possibility of carrying out comparative studies and searches for pathways involved in the complex process of somatic embryogenesis that will allow for a better understanding of the mechanism that drive somatic embryogenesis in higher plants.

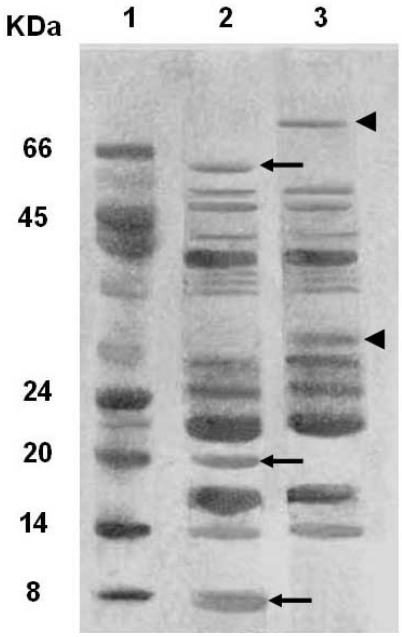

Fig. 2 SDS-PAGE profile of intracellular proteins from carnation embryogenic and non-embryogenic callus. Lane 1: marker proteins; lane 2: embryogenic callus; lane 3: non-embryogenic callus. Numbers on the left = reference marker protein (Roche Molecular Biochemicals) size (in kDa). Arrows indicate the specific proteins in embryogenic callus and arrowheads indicate the specific proteins in non-embryogenic callus.

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