Research Note

Immunodetection of chitinase-like and β-1,3-glucanase-like proteins secreted *in vitro* by embryogenic and non-embryogenic cells of grapevines

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Summary: Embryogenic and non-embryogenic cell lines were obtained from the rootstock 41B (*Vitis vinifera* cv. Chasselas x *V. berlandieri*). A few extracellular proteins from culture media of embryogenic and non-embryogenic grapevine cell lines during multiplication showed a positive reaction with anti-chitinase antibodies (embryogenic cell lines) and anti-β-1,3-glucanase antibodies (non-embryogenic cell lines).

Key words: grapevine somatic embryogenesis, chitinase, β-1,3-glucanase, Western blotting.

Introduction: The genetic transformation of grapevine by *Agrobacterium tumefaciens* is one way to protect grapevines against diseases. This technology requires an efficient regeneration system after cell transformation to obtain genetically improved plants (Martinelli *et al.* 1993; Le Gall *et al.* 1994; Mauro *et al.* 1995). The study of intra- and extracellular proteins of cells by *in vitro* culture would enlarge our understanding of the ability of plants for cell multiplication, development and regeneration (De Vries *et al.* 1988; Coutos-Thevenot *et al.* 1992; Domon *et al.* 1994).

Recent results suggest both, a structural role and a developmental function of proteins which are involved in defense mechanisms of plants ('pathogenesis-related proteins', PRs), as described by Van Loon *et al.* 1994). We have compared patterns of secreted proteins from grapevine cell suspension culture media (containing NOA) of embryogenic and non-embryogenic cell lines at the multiplication stage. We focused on PRs-like proteins detected by antibodies raised against two families of tobacco PR-proteins: β-1,3-glucanase (PRs-2) and chitinase (PRs-3).

Materials and methods: Different sources of explants of the rootstock cultivar 41B (*Vitis vinifera* cv. Chasselas x *V. berlandieri*) were used to produce initial calli. Embryogenic cell lines (E-cells) were established from diploid tissue deriving from anther culture whereas non-embryogenic cell lines (NE-cells) originated from leaves of *in vitro* plantlets (Deloire, unpubl. data). For multiplication of E-cell and NE-cell lines, subcultures were carried out in liquid media, containing 5 mM 2-naphthoxyacetic acid (NOA), at 22 °C in the dark, according to Mauro *et al.* (1995). Proteins secreted in liquid media were extracted following the method described by Coutos-Thevenot *et al.* (1992).

SDS-PAGE electrophoresis was performed using the minislab gel apparatus mini Proteans II (Bio-Rad, Richmond, CA) and the method of Laemmli (1970). Immunological detection was performed according to Thiriet and Albert (1995). Antisera of rabbits were used (provided by B. Fritig, CNRS, Strasbourg), which were raised against the tobacco PRs-2 (β-1,3-glucanase) and PRs-3 (chitinase) families (Kauffmann *et al.* 1987; Legrand *et al.* 1987). The immuno precipitates were detected using a peroxidase conjugated anti-rabbit IgG revealed by diaminobenzidine.

Results: Different kinds of cell growth and subsequent development according to the origin of the cultures were observed. Only E-cells regenerated to embryos when the cells were transferred to the medium (Coutos-Thevenot *et al.* 1992 b) without phytohormone, unlike NE-cells which grew unorganized comparable to the growth of undifferentiated calli cells.

A comparison of Western blottings of extracellular proteins showed a clear difference between lines of E- and NE-cells at the multiplication stage. Anti-chitinase (anti-Q) antiserum reacted only with proteins secreted into the E-cell line culture media during multiplication and developmental stages (bands around 34 kDa) from 10-d-old cultures. No reaction was detected with the secreted proteins from NE-cells (Fig. 1). In contrast, anti-β-1,3-glucanase antisera (anti-2 and anti-gluc b) showed reactions only with proteins secreted from NE-cell lines (bands around 35 kDa), from 4-, 7- and 10-d-old cultures. No reaction was detected with proteins secreted from the E-cell lines (Fig. 2).

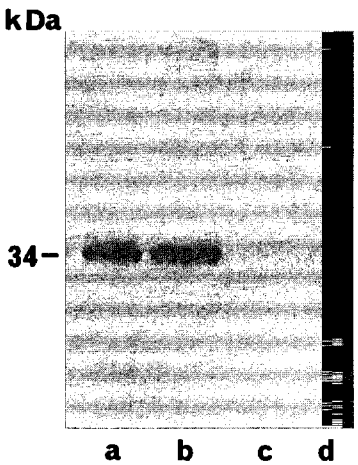

Fig. 1: Western blot of secreted proteins from embryogenic cells at the multiplication stage (a) and at the developmental stage (b). The antiserum used is raised against tobacco PR-3 chitinase (Anti-Q). A 34 kDa chitinase-like protein band is detected among extracellular proteins in both stages (a, b). No band was detected among extracellular proteins of non-embryogenic cells at the multiplication (c) and at the developmental stages (d).

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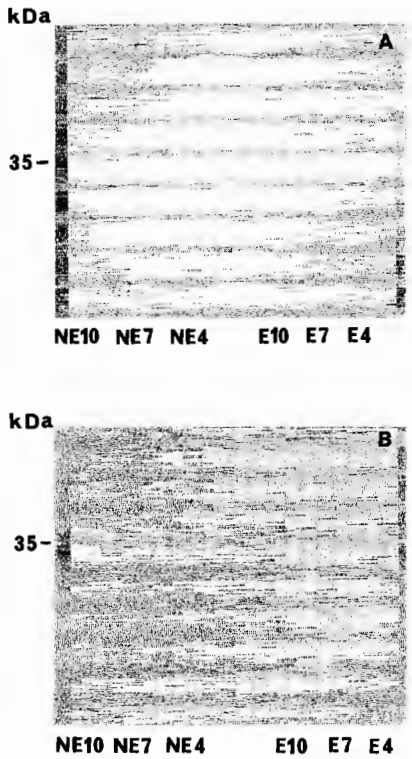

Fig. 2: Western blots of secreted proteins from embryogenic cells (E; 4, 7 and 10 days) and non-embryogenic cells (NE; 4, 7 and 10 days). The antisera used are raised against tobacco PR-2, β-1,3-glucanase (anti-2 and anti-gluc b). The 35 kDa β-1,3-glucanase-like protein bands were detected among extracellular proteins of non-embryogenic cells with both, anti-2 (**A**) and anti-gluc b (**B**) antisera. No band was detected among secreted proteins from embryogenic cells (E; 4, 7 and 10 days).

Discussion: The embryogenic and non-embryogenic grapevine cell lines incubated under the same culture conditions differed in their total pattern of secreted proteins (KRAEVA, unpubl. data). Among the released proteins, chitinase and β-1,3-glucanase activities were detected in different culture systems, both in embryogenic and non-embryogenic cultures (ESAKA *et al.* 1990; GAVISH *et al.* 1991). For the first time, we have shown with immunological techniques a) the presence of chitinase-like proteins among extracellular proteins of embryogenic cell lines and b) the presence of β-1,3-glucanase-like proteins among extracellular proteins in non-embryogenic cell lines in grapevines.

In the absence of a known chitinase substrat (i.e. chitin) in plant cells, we may raise the question whether chitinase plays a role in plants multiplication or development. RÖHRIG *et al.* (1995) have considered that lipochitooligosaccharides (LCOs), which may be present in plant cell walls (or homologous structures), are hydrolysed by chitinase. In tobacco protoplast cultures, the absence of phytohormones was compensated by the addition of synthetic LCOs. The authors conclude that LCOs, which are hydrolysed by chitinase, can be considered as plant growth regulators. We have shown a correlation between extracellular chitinase-like proteins and embryogenic cells in a perennial plant. We pursue this study with grapevine homologous chitinase and glucanase probes and/or antibodies, comparing intra- and extracellular cell proteins *in vitro*.

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