BIOLOGIA PLANTARUM 39 (1): 125-130, 1997

# Biochemical variation between non-embryogenic and embryogenic calli of silver fir

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## Abstract

Comparative study on SDS-protein profiles and isoenzyme composition of non-embryogenic and embryogenic calli in two callus lines of silver fir *(Abies alba* Mill.) revealed the presence of abundant polypeptide fractions and an increased number of isoperoxidases in non-embryogenic calli. Non-specific esterase, on the other hand, exhibited an opposite tendency, while glutamate dehydrogenase was the only enzyme system consisting uniformly of one isoenzyme band in both types of calli investigated.

*Additional key words*: *Abies alba*, isoenzymes, proteins.

## Introduction

The embryogenic and non-embryogenic calli of conifers have been shown to differ not only in coloration and consistency but also biochemically. Wann *et al.* (1989) have for example illustrated different isoperoxidase composition of these types of calli in *Picea abies*. At the polypeptide level, a high degree of 84 kD polypeptide synthesis was ascribed specifically to the embryogenic callus, with on abundancy of 55 and 18 kD polypeptides in the non-embryogenic callus of this species (Norgaard 1988). Non-specific esterase, malate dehydrogenase and glutamate dehydrogenase are additional enzyme systems whose association with embryogenic activity of a callus has been demonstrated experimentally in a variety of plant species (Fransz 1988, Joersbo *et al.* 1989, Rao *et al.* 1990). It is believed that some of these enzymes may function as markers of somatic embryogenesis, revealing those steps in differential gene activity which govern the regeneration potential of embryogenic callus.

*Received* 20 September 1995, *accepted* 14 November 1995.

*Abbreviations*: BAP - 6-benzylaminopurine; GDH - glutamate dehydrogenase; NAA - α-naphthyl-acetic acid; PAGE - polyacrylamide gel electrophoresis; SDS - sodium dodecyl sulphate.

## Materials and methods

Embryogenic and non-embryogenic calli of *A. alba* Mill. callus lines no. 12/2 and 13/2 were initiated from one immature embryo of each. The Schenk and Hildebrandt (1972) medium (SH) used for induction of both types of calli was supplemented with 6-benzylaminopurine (BAP) and α-naphthylacetic acid (NAA) (1 mg $dm^{-3}$ of each) in the case of non-embryogenic calli and with BAP only (1 mg $dm^{-3}$) in the case of embryogenic calli (Vooková *et al.* 1992).

For protein extraction, 1 g of callus tissue and 1.5 $cm^3$ of extraction buffer were used (Pitel *et al.* 1992). The tissue of each callus line was selected randomly from 2 - 3 pieces of callus in two different plates. Following centrifugation of the homogenates at 13 000 *g*, the aqueous supernatants of individual samples were obtained, desalted on *Sephadex G 25* column and loaded on the gels at a volume of 0.08 $cm^3$ of each.

Disc polyacrylamide gel electrophoresis was conducted according to Fric (1971). Benzidine, α-naphthylacetate and neutralized L-glutamic acid were used as substrates in the visualization of peroxidases, non-specific esterases and glutamate dehydrogenases, respectively.

For the purpose of SDS-polyacrylamide electrophoresis, the callus tissue was homogenized in 0.125 M Tris HCl extraction buffer, pH 6.8, containing 22.5 % (v/v) mercaptoethanol, 22.5 % (v/v) glycerol and 9 % (m/v) SDS, respectively. The crude homogenate was heated for 3 min at 95 °C, clarified by centrifugation at 15 000 *g* for 10 min and subjected to SDS-PAGE (Laemmli 1970).

## Results

The comparative biochemical study on non-embryogenic and embryogenic calli of callus culture lines 12/2 and 13/2 revealed various differences in their SDS protein profiles and isoenzyme composition. The non-embryogenic calli possessed some polypeptide fractions which were missing in embryogenic calli. The polypeptide spectrum of non-embryogenic calli of line 12/2 was enriched by the components with relative mobility 0.32, 0.36, 0.56, 0.63 and 0.75, respectively, which were not detected in embryogenic calli of the line (Fig. 1*a-e*). The same applies for the non-embryogenic and embryogenic calli of line 13/2 which did not however differ so profoundly, deviating only in the polypeptide fraction of relative mobility 0.75 (Fig. 1*f-i*).

Among the enzyme systems tested so far, that of peroxidase was found to be the most heterogeneous, reflecting very sensitively not only the metabolic differences between non-embryogenic and embryogenic calli of a given line but also the biochemical variation between individual lines. An increased number of peroxidases in non-embryogenic calli of both lines (Fig. 2*a,b,e,f,*) contrasted with the reduced level of isoenzymes in embryogenic calli, represented by one band only in line 12/2 (Fig. 2*c,d*) as well as by the three enzymatically active fractions in line 13/2 (Fig. 2 *g,h*).

A somewhat analogous situation was typical also for a non-specific esterase, which exhibited a higher number of molecular forms in non-embryogenic calli of line

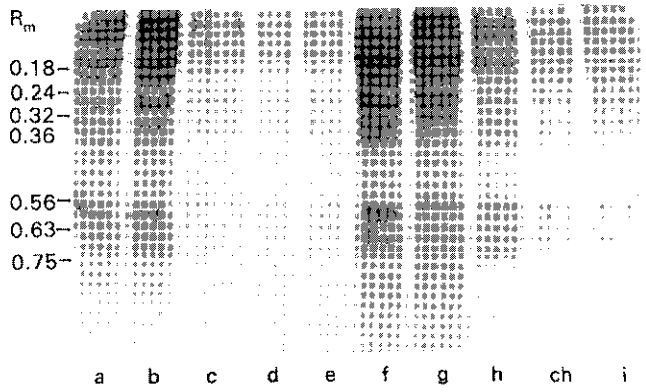


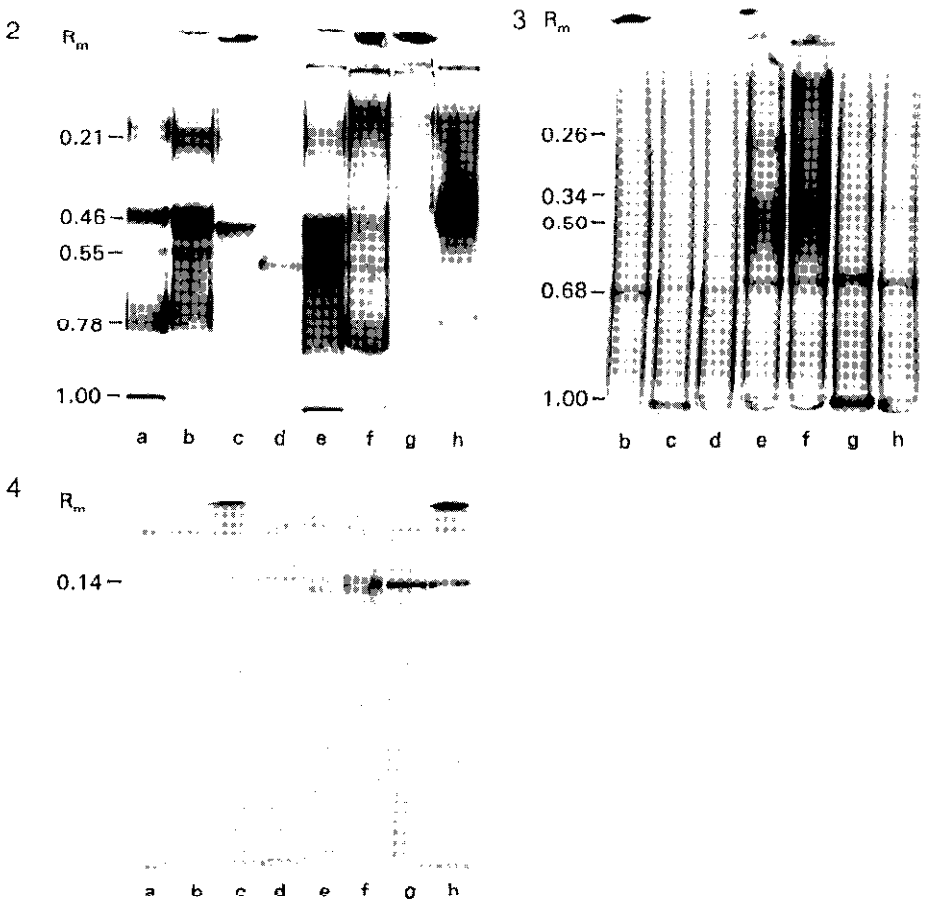

Fig.1. SDS protein profiles of non-embryogenic and embryogenic calli of silver fir callus culture lines 12/2 and 13/2. *a, b* - 12/2 non-embryogenic calli; *c, d, e* - 12/2 embryogenic calli; *f, g* - 13/2 non-embryogenic calli; *h, ch, i* - 13/2 embryogenic calli

no.13/2 than in embryogenic tissues (Fig. 3*e-h*). The reverse situation was however typical for line 12/2, the embryogenic calli of which were enriched by one isoesterase band (Rm 1.0) not revealed in non-embryogenic callus (Fig. 3*b-d*). The enzyme glutamate dehydrogenase was exceptional in this respect, consisting uniformly of only one isoenzyme fraction in all kinds of the calli investigated. The only differences observed so far concerned the intensity of visualized bands indicating quantitative rather than qualitative differences between non-embryogenic and embryogenic calli. Only a trace of glutamate dehydrogenase activity was detected in non-embryogenic calli of both callus culture lines (Fig. 4*a,b,e,f*), contrasted with more distinct bands of the enzyme in embryogenic calli (Fig. 4*c,d,g,h*)

## Discussion

The transition of non-embryogenic callus with unorganized growth into embryogenic callus with typically aggregated globular clusters of cells represents the initial phase of somatic embryogenesis in most plant species and is accompanied by profound metabolic differentiation (Nomura and Komamine 1986). Wann *et al.* (1987) have illustrated significant differences between embryogenic and non-embryogenic calli of *Picea abies* in ethylene evolution rate as well as in concentrations of glutathione and total reductans. A high frequency and synchronous embryogenesis system achieved in silver fir (Vooková *et al.* 1992) has enabled the analysis of this process also in terms of isoenzyme composition of calli, with special reference to those enzyme systems which are postulated to have association with the process of somatic

embryogenesis. In particular it is true of peroxidase has been shown to function as a biochemical marker of somatic embryogenesis in *Daucus carota* (Joersbo *et al.* 1989), *Zea mays* (Fransz 1988) and *Citrus sinensis* (Kochba *et al.* 1977). The isoperoxidase profiles of all these species, with a higher number of isoenzymes




Figs. 2 - 4. Isoperoxidase (*2*), non specific esterase (*3*) and glutamatedehydrogenase (*4*) compositions of non-embryogenic (*a,b,e,f*) and embryogenic calli (*c,d, g,h*) of callus culture lines 12/2 (*a-d*) and 13/2 (*e-h*).

revealed in non-embryogenic calli rather than in embryogenic ones, have also been shown here by the corresponding profiles of *A. alba* calli, which exhibited the same tendency. It is our impression that as well as the physiological reasons of an increased peroxidase activity in non-embryogenic calli due to a higher content of phenolic substances causing the browning of callus tissues, the revealed differences reflect also a close relationships between the isoperoxidase complements of embryogenic calli and their morphogenetic potentials. The same type of differences

in isoperoxidase banding patterns in coniferous species has also been reported by Wann *et al.* (1989).

Contrary to isoperoxidases, the GDH behaved in a different way, exhibiting negligible or only very low activity in non-embryogenic calli and a more intensively stained band in embryogenic calli of both callus lines investigated. Regarding the metabolic role and physiological significance of glutamate dehydrogenase in the biosynthetic processes of a cell, we may only speculate that this increment in enzyme activity of embryogenic calli is related to their growth and morphological potentials which are incomparably higher than the corresponding processes in non-embryogenic calli.

In contrast with peroxidase and glutamate dehydrogenase, the isozyme pattern of non-specific esterase exhibited an opposite tendency in the two lines compared, which complicates any more detailed specification of the role which the enzyme plays during the initial phase of somatic embryogenesis in silver fir. Only seems unequivocal the significance of peroxidase in discriminating between non-embryogenic and embryogenic calli of the species.

As to the protein composition of calli, it seems reasonable to relate the absence of embryogenic activity in non-embryogenic calli to the abundant polypeptide fractions present in their SDS protein profiles. These fractions represent the storage proteins that in developing zygotic embryos appear during the early cotyledonary stage and which were proved to affect adversely the morphogenetic competence of zygotic embryos in conifers (Flinn *et al.* 1991, Hakman 1993). The two immature zygotic embryos which gave rise to non-embryogenic calli seem to have reached this stage of their development either at the moment of their collection or after transfer to the induction medium. Alternatively, we may suppose that embryogenic calli derived from the non-embryogenic calli mentioned above are the result of hormonally suppressed proteosynthesis of the latter.

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