VI.6 Isozymes as Markers for Embryogenic Maize Callus

J.H.N. Schel, N.C.A. de Ruijter, and P.F. Fransz

1 Introduction

The search for markers of plant embryogenesis is an important aspect of modern plant breeding and isozyme analysis remains a powerful tool to register cyto-differentiation in plants. The use of in situ cytochemical techniques to study the proteins which are expressed during embryogenesis has been underestimated so far. This chapter, mainly based on our earlier work (Fransz et al. 1989a,b), emphasizes the possibilities and applications of combined biochemical and cytochemical studies. Besides giving a survey of the literature about isozymes as biochemical markers during embryogenesis, especially for maize, it accentuates the use of cytochemical techniques to study the in situ expression of these isozymes.

2 Isozymes During Plant Embryogenesis

The expression of the differentiation of cells, as reflected by their isozyme patterns, may be used to obtain a better understanding of the basic mechanisms of cellular differentiation and further plant development. For maize, three enzymes have particularly proven to be very suited to study these developmental pathways, i.e., amylase, catalase and alcohol dehydrogenase. Amongst them, catalase, used as a model system, was found to be coded by three genes, giving rise to various isozyme patterns, especially during kernel development and seed germination. The isozyme pattern of alcohol dehydrogenase in maize scutella shows two forms of ADH, designated as ADH-1 and ADH-2 (see chap. VI.5, this vol.). At the onset of kernel development, two additional isozymes emerge, being most prominent in the latest stages of embryo development and during the first days of germination of the dry seeds (Scandalios 1974; see Fig. 1; for a survey, see Goodman and Stuber 1983).

These developmental changes have prompted investigators in the last decade to examine the usefulness of isozymes as markers not only for the later stages of the growing plant, e.g., organogenesis or seed germination, but also for the early

1 Department of Plant Cytology and Morphology, Agricultural University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands
Isozymes as Markers for Embryogenic Maize Callus

Fig. 1. A composite schematic zymogram of the developmental pattern of the alcohol dehydrogenase isozymes in maize scutella. A endosperm 16–30 days after pollination (for comparison); B scutellum 16–33 days after pollination (pattern is same whether single or pooled scutella are assayed); C scutellum 34–39 days after pollination; D scutellum 45–49 days after pollination; E scutellum 50–56 days after pollination; F scutellum from dry dormant seed; G scutellum from imbibed seed; H, I, J, K, L are respectively, scutella from 1, 2, 3, 4, and 10 days after germination (sporophytic development). 0, point of sample insertion; migration is anodal. (Scandalias 1974)

development of the plant, i.e., during early embryogenesis. These studies became possible because the relatively large amounts of material needed for sample preparation and electrophoresis are present by in vitro culture of plant tissues and subsequent somatic embryogenesis. Defined stages of embryo development can be obtained in high quantities from synchronized cultures (for a review, see, e.g., Ammirato 1983).

We have to keep in mind the specific problems which may be encountered during analysis of isozyme patterns from tissue cultures. In general, there seems to be much variation in callus cultures, as was shown for ADH in carrot cultures (Chourely and Widholm 1980) and for peroxidases and polyphenoloxidases from the eggplant (Del Grosso and Alicchio 1981). Also, Lo Schiavo et al. (1980) demonstrated qualitative differences in isozyme patterns obtained from leaf and callus extracts of several species of Nicotiana. In spite of these drawbacks, several studies have appeared in the past years describing biochemical differences between embryogenic and nonembryogenic plant tissues.

Wann et al. (1987) found for Picea abies callus significant differences in the evolution rate of ethylene and in the concentrations of glutathione and total reductant. However, they did not perform comparative electrophoretic studies. For pea callus such a study was carried out by Stirn and Jacobsen (1987). Alterations of polypeptide pattern were investigated using two genotypes of Pisum sativum. One type gave rise to a yellowish callus, forming somatic embryos in suspension culture; the other type produced a white compact callus without regenerative capacity. Using semi-preparative two-dimensional electrophoresis, IEF combined with SDS-PAGE, these authors detected two protein bands at 45 and 70 kDa which were characteristic for the embryogenic callus line and were very similar to proteins found in carrot embryogenic cultures (Sung and Okimoto 1981). Also for carrot cultures, more recently two major groups of esterase isozymes were found to differ in an embryogenic cell line as compared with a non-embryogenic line (Chibbar et al. 1988).