Contrasting pattern of somatic and zygotic embryo development in alfalfa (Medicago sativa L.) as revealed by scanning electron microscopy

Nanfei Xu and J. Derek Bewley

Department of Botany, University of Guelph, Guelph, Ontario N1G 2W1, Canada

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Abstract. Scanning electron microscopy has been used to investigate the morphological changes occurring during the development of alfalfa somatic embryos. Embryos were initiated from callus, transferred to suspension culture and matured on solid agar medium. This developmental pattern was compared to that of zygotic embryos developing in ovulo. Somatic embryos begin as distinct pro-embryos within the callus tissue pieces placed in suspension culture. They become globular and heart-shaped while on solid agar medium and then undergo cotyledon elongation and maturation. Somatic embryos develop comparatively slower at early stages of development and faster at the later stages than zygotic embryos. They lack a well-defined suspensor and have a very rough, poorly-differentiated epidermis, the first layer of which is lost after pro-embryo formation. The cotyledons of somatic embryos are multiple and poorly-developed; there appears to be a correlation between the amount of surface roughness of the developing embryo and the extent to which polycotyledony occurs.

Key words: Medicago sativa, embryogenesis, somatic embryo, scanning electron microscopy.

Introduction

Somatic embryogenesis from plant tissue culture was first recognized by Steward et al. (1958) and Reinert (1958, 1959) and has since been achieved for many species (Ammirato, 1983). Several different vegetative tissues have been used to produce somatic embryos, which develop in a similar way to zygotic embryos, and they can germinate into viable seedlings. Recent improvements in tissue culture techniques have made it possible to obtain desiccation-tolerant somatic embryos (Gray et al., 1987; Senaratna et al., 1990) which, like most orthodox seeds, can undergo dehydration without loss of viability. These successes provide both good material for studies of plant embryogenesis and a promising future for artificial seed production. However, somatic embryos are frequently unlike their zygotic counterparts, and morphological abnormalities are commonplace (Ammirato, 1983). The major storage structures in zygotic alfalfa embryos are the cotyledons, which are well developed and contain starch and storage proteins (Krochko and Bewley, 1988; Coulter and Bewley, 1990). In alfalfa somatic embryos, the most obvious abnormality is the poorly developed, short and often branched or multiple cotyledons. This poor development is accompanied by a reduced deposition of storage reserves, such as proteins (Stuart et al., 1985, Krochko et al., 1991). The cause of this abnormality remains unknown.

While direct somatic embryogenesis from callus tissue has been reported in alfalfa (Saunders and Bingham, 1972; Kao and Michayluk, 1980; Santos et al., 1980; Johnson et al., 1981), the most practical method of producing alfalfa somatic embryos is via suspension culture (Senaratna et al., 1989; Senaratna et al., 1990; Fujii et al., 1990; Anandarajah and McKersie, 1990a; Anandarajah and McKersie, 1990b). The origin and development of alfalfa somatic embryos from suspension culture has not been characterized. In this study, the morphological changes during somatic embryogenesis of alfalfa are followed from callus initiation to the cotyledon elongation stage of embryo development using scanning electron microscopy. Since poor cotyledon development is a feature of somatic embryogenesis in this species, particular emphasis was paid to cotyledon initiation. For comparative purpose, morphological changes occurring during zygotic embryogenesis are outlined also.

Materials and methods

The growth of alfalfa (Medicago sativa L.) cv. Excalibur plants and staging of zygotic embryos during their development was according to Xu et al. (1991). Dissection of the zygotic seeds was according to Sangduen et al. (1983a). Somatic embryos were grown by the methods of Senaratna et al. (1990) and were collected on a daily basis. The zygotic embryos and somatic embryos were fixed according to Brown and Greenwood.
Figure 1. Scanning electron micrographs of alfalfa zygotic embryos. A, early globular stage; B, middle globular stage; C, late globular stage; D, early heart stage, showing cotyledon initiation; E, late heart stage, showing the beginning of cotyledon elongation; F, torpedo stage (note the formation of radicle); G, cotyledon elongation stage; H, zygotic embryos with four cotyledons. Bar = 30 μM.

(1990) and post-fixed in 2% osmium tetroxide solution. They were then dehydrated in a graded ethanol series, solvent exchanged with liquid CO₂, critical-point dried, sputter-coated with gold-palladium and examined under JEOL JSM 35C scanning electron microscope.

Results and discussion

Development of zygotic embryos
Zygotic embryogenesis in alfalfa has been studied morphologically and physiologically by a number of researchers (e.g. Martin, 1914; Reeves, 1930a, 1930b; Cooper, 1935; Cooper et al, 1937; Sangduen et al, 1983a, 1983b; Xu et al, 1990), although this is the first study of the embryo developmental stages using scanning electron microscopy. The youngest embryos obtained by dissection of the ovule are those at the globular stage (Fig. 1). The early globular stage embryo is oval-shaped (Fig. 1A), and as it continues to grow it changes into a round mass of cells (Fig. 1B). The flattening of the top of globular embryo (Fig. 1C) is followed by cotyledon initiation (Fig. 1D), which marks the beginning of heart stage of development. Late heart stage is reached when the cotyledon length about 10 μm (Fig. 1E). Following cotyledon elongation there is the formation of the radicle, then the embryo assumes a characteristic torpedo shape (Fig. 1F). Further growth results in curvature of the embryo as its length exceeds the width of the seed (Fig. 1G).

Attached to the alfalfa zygotic embryo is a conspicuous suspensor (Fig. 1A to 1E). An early study of the structure of this suspensor lead to the claim that it is composed of only one rank of cells (Martin, 1914). However, in 1983, Sangduen et al. showed in a TEM study that the suspensor