Histology of somatic embryogenesis in mature tissues of olive (Olea europaea L.)

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(Accepted 7 October 2000)

SUMMARY
This anatomical investigation on olive secondary somatic embryos describes several aspects of embryo development, including preembryoid origin and growth, aspects of tissue differentiation, localization of somatic embryogenesis, and starch occurrence. The failure of a number of secondary somatic embryos to develop into perfect structures is to be ascribed to defects in the last growth stages (fused embryos, fused cotyledons) and/or to tissue degeneration processes affecting both imperfect and apparently perfect somatic embryos.

Somatic embryogenesis, until recently possible with only a few tree species, is rapidly becoming a common regeneration technique for several woody plants, due to the huge increase of knowledge on physiological and biochemical factors controlling its induction and maintenance, as well as the subsequent maturation and development of somatic embryos. Moreover, somatic embryogenesis is an excellent morphogenetic system which permits the investigation of differentiation processes. Unlike zygotic embryogenesis, a profusion of somatic embryos can easily be obtained from an embryogenic line maintained under controlled external conditions (as regards medium composition, light, photoperiod, etc.). The extensive literature in this field has also considered the histological aspects of the initiation and development of somatic embryos; however, several fundamental aspects of the process remain unclear or appear to differ among the systematic groups.

The main issues still attracting the attention of researchers are: the origin of somatic embryos, both as concerns the primary tissues (Alemanno et al., 1996; Lee et al., 1997; Chan et al., 1998; Samantaray et al., 1997; Kemper et al., 1996; Puigderrajols et al., 1996; Rout and Lucas, 1996; Rout et al., 1998) and whether the origin is unicellular or multicellular (Alemanno et al., 1996; Loiseau et al., 1998; Lee et al., 1997; Marin Hernandez et al., 1998; Nonohay et al., 1999; Puigderrajols et al., 1996); cytological aspects of the competent cells (Alemanno et al., 1996; Lu and Vasil, 1995; Menendez-Yuffà and Garcia de Garcia, 1997); histochemistry of the somatic embryos in the course of their formation and development, particularly as concerns starch, proteins, polyphenols (Goh et al., 1999; Alemanno et al., 1996; Marin Hernandez et al., 1998; Svodobovà et al., 1999; Garin et al., 1997; Loiseau et al., 1998; Navarro et al., 1997), just to mention the most recent literature.

For the olive (Olea europaea L.) an efficient protocol of somatic embryogenesis is available, both from immature zygotic embryos (Rugini, 1988), and from mature tissue of a cultivar (Rugini and Caricato, 1995), which proved to be a promising regeneration system for different biotechnological applications (Lambardi et al., 1999; 2000). In the latter system, primary somatic embryos are originated from morphogenetic masses, produced from the leaflets of in vitro growing shoots of the cv Canino. Up to now this method, called by Rugini and Caricato (1995) “double regeneration system”, is the only one allowing the induction of somatic embryogenesis in mature tissues of olive. By subculturing primary somatic embryos onto an appropriate induction medium it is possible to produce secondary somatic embryos for hundreds of subcultures. However, the high number of somatic embryos that fail to develop into healthy plants still represents a major obstacle, preventing a further optimization of the technique. This research was an attempt to shed more light on the histological aspects of somatic embryogenesis in the olive, with a view to describing the process and to find histological explanations of abnormalities of somatic embryos that are unable to complete their development.

MATERIALS AND METHODS
Induction and maintenance of embryogenic lines
Four year old morphogenetic masses of cultivar Canino, produced by the “double regeneration system”, described by Rugini and Caricato (1995), were maintained at 23±1°C in the dark by monthly subcultures in hormone-free OMc (Canas et al., 1991) medium with 0.1% activated charcoal added. Primary somatic embryos, differentiated from these masses, were carefully isolated with forceps at different stages of development (from the torpedo up to the cotyledonal stage) and subcultured onto the same medium, to induce the formation of secondary somatic embryos, directly from the surface of the primary somatic embryos.