

ORGANOGENESIS FROM INTERNODE-DERIVED NODULES OF *HUMULUS LUPULUS* VAR. NUGGET (CANNABINACEAE): HISTOLOGICAL STUDIES AND CHANGES IN THE STARCH CONTENT¹

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The sequence of histological and histochemical events occurring during organogenesis from *Humulus lupulus* var. Nugget internode-derived nodules was studied. Sections were made and studies were carried out from the start of culture treatment until the development of shoot buds. Cell division was observed in both cambial and cortical regions during the first week of culture establishment. Cell division in cortical cells led to the formation of an incipient callus tissue. From the calluses prenodular structures of cambial origin appeared and gave rise to nodules from which shoot buds formed. Nodules kept separating into "daughter nodules" from which arose an increasing number of shoot buds. Iodide staining showed a strong starch accumulation in callus tissue and in prenodular structures. During shoot-bud primordia formation starch content decreased in nodules. Some starch was also noted in control explants (cultured on basal medium), however at a lower level than that observed in explants cultured on media with growth regulators. Shoot-bud regeneration was not observed in control explants.

Key words: Cannabinaceae; histological studies; *Humulus lupulus* var. Nugget; internodes; nodules; organogenesis; starch.

The totipotent character of plant cells and tissues can be expressed by their ability to regenerate into plants via embryogenesis or organogenesis. Both processes lead to in vitro regeneration and are a major prerequisite for genetic transformation. However, the widespread application of gene transfer techniques for crop improvement cannot be successfully achieved if the processes leading to morphogenesis are not well understood.

Histological, histochemical, and ultrastructural aspects of somatic embryogenesis, whether occurring with or without a callus phase, have been extensively reported (Ho and Vasil, 1983; Williams and Malleshwaran, 1986; Stamp, 1987; Pedroso and Pais, 1993; Brisibe et al., 1993; Sagare, Subasini, and Krishnamurthy, 1995). A system of efficient regeneration parallel to somatic embryogenesis has been recently proposed (Aitken-Christie, Singh, and Davies, 1988; McCown et al., 1988; Warrag, Lesney, and Rockwood, 1991; Teng, 1997) and is based on the formation of organogenic nodules that arise directly from the explants or from callus and cell suspension cultures.

Morphogenesis requires an external source of carbon, which is usually supplied in the medium mainly as sucrose. Thorpe and Murashige (1970) and Mangat, Pelekis, and Cassels (1990) have shown that in tobacco callus and in *Begonia rex* stem explants there is a strong accumulation of starch in areas that eventually give rise to meristemoids and shoot primordia in tissues grown on shoot-forming media. High amounts of starch have also

been observed during somatic embryogenesis in sugar cane and cassava (Ho and Vasil, 1983; Stamp, 1987). The possible role of starch in these processes is not clear. It has been suggested that starch may function as an energy source or may provide osmoticity in the form of free soluble sugars (Thorpe, 1980; Stamp, 1987). Regardless of its function, starch accumulation/mobilization cycles appear to be a characteristic feature of cells involved in plant morphogenesis.

This paper reports on the histological events leading to nodule formation and shoot regeneration from internode-derived nodules of *Humulus lupulus* var. Nugget and on the evidence of a starch accumulation/mobilization cycle during this morphogenic process.

MATERIALS AND METHODS

Tissue culture.—Internodal explants were taken from 3–4 mo old plants micropropagated in Adams (1975) media. Explants were first cut into pieces 6–9 mm long and then inoculated in culture flasks (9 × 8 cm) containing ~40 mL of MS solid medium (Murashige and Skoog, 1962) supplemented with 2 mg/L BAP, 0.05 mg/L IAA, 18 g/L sucrose, and 7.8 g/L agar (Vas Pereira, Lisbon, Portugal). Control explants were cultured as described above but on medium without growth regulators. The pH was adjusted to 5.7–5.8 before autoclaving at 121°C and 1.01 × 10⁵ Pa for 18 min. All cultures were incubated at 25° ± 2°C with a 16-h photoperiod (35 μmol photons·m⁻²·s⁻¹) provided by cool-white Philips fluorescent tubes.

Histology and histochemistry.—Cultured explants were removed at random at 0, 2, 5, 7, 9, 12, 15, 19, 23, 28, 31, 35, and 45 d and fixed in 30% formalin:glacial acetic acid:70% ethanol (1:1:18) for 72 h at 4°C. Control explants were removed after 8, 15, 28, and 45 d in culture on MS medium without growth regulators and fixed as described above. Sections (20–40 μm) of the fixed internodal segments were obtained

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Current Advances in Fern Research Helena Fernández, 2018-03-24 Ferns collectively represent an ancient species of vascular plant which has a direct connection to the beginning of life on Earth Today they are valued for their ornamental appeal environmental benefit or as sources of health benefiting metabolites Current pteridology the study of fern encompasses a wide range of research activities including but not limited to plant physiology stress tolerance genetics and genomics The goal of this book is to compile the most relevant research done with ferns during the last decade It is organized into four parts I Biology and Biotechnology II Evolution and Conservation III Metabolism and Genetic Resources and IV Environment Each section reveals the utilization of ferns as a tool to explore challenges unique to plant development and adaptation This project represents our collective effort to raise the awareness of ferns as a model system to study higher plant functions Among the distinctive features of our proposed book are i a wide range of topics with contributing researchers from all around the world and ii recent advances of theoretic and applied knowledge with implications to crop species of economic value **American Journal of Botany** ,2000 Australian Journal of Botany ,2010 **Memoirs of the Scientific Sections of the Academy of the Socialist Republic of Romania** ,2003 *Chemical Abstracts* ,2002 **Pastos y forrajes** ,2004 **Bibliography of Agriculture** ,1996

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