

## ORGANOGENESIS FROM INTERNODE-DERIVED NODULES OF *HUMULUS LUPULUS* VAR. NUGGET (CANNABINACEAE): HISTOLOGICAL STUDIES AND CHANGES IN THE STARCH CONTENT<sup>1</sup>

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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 Malleshwarum, 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 × 10<sup>5</sup> Pa for 18 min. All cultures were incubated at 25° ± 2°C with a 16-h photoperiod (35 μmol photons·m<sup>-2</sup>·s<sup>-1</sup>) 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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# Organogenesis From Internode Derived Nodules Of Humulus

**EW Minium**



## **Organogenesis From Internode Derived Nodules Of Humulus:**

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### **Table of Contents Organogenesis From Internode Derived Nodules Of Humulus**

1. Understanding the eBook Organogenesis From Internode Derived Nodules Of Humulus
  - The Rise of Digital Reading Organogenesis From Internode Derived Nodules Of Humulus
  - Advantages of eBooks Over Traditional Books
2. Identifying Organogenesis From Internode Derived Nodules Of Humulus
  - Exploring Different Genres
  - Considering Fiction vs. Non-Fiction
  - Determining Your Reading Goals
3. Choosing the Right eBook Platform
  - Popular eBook Platforms
  - Features to Look for in an Organogenesis From Internode Derived Nodules Of Humulus
  - User-Friendly Interface
4. Exploring eBook Recommendations from Organogenesis From Internode Derived Nodules Of Humulus
  - Personalized Recommendations
  - Organogenesis From Internode Derived Nodules Of Humulus User Reviews and Ratings
  - Organogenesis From Internode Derived Nodules Of Humulus and Bestseller Lists

5. Accessing Organogenesis From Internode Derived Nodules Of Humulus Free and Paid eBooks
  - Organogenesis From Internode Derived Nodules Of Humulus Public Domain eBooks
  - Organogenesis From Internode Derived Nodules Of Humulus eBook Subscription Services
  - Organogenesis From Internode Derived Nodules Of Humulus Budget-Friendly Options
6. Navigating Organogenesis From Internode Derived Nodules Of Humulus eBook Formats
  - ePub, PDF, MOBI, and More
  - Organogenesis From Internode Derived Nodules Of Humulus Compatibility with Devices
  - Organogenesis From Internode Derived Nodules Of Humulus Enhanced eBook Features
7. Enhancing Your Reading Experience
  - Adjustable Fonts and Text Sizes of Organogenesis From Internode Derived Nodules Of Humulus
  - Highlighting and Note-Taking Organogenesis From Internode Derived Nodules Of Humulus
  - Interactive Elements Organogenesis From Internode Derived Nodules Of Humulus
8. Staying Engaged with Organogenesis From Internode Derived Nodules Of Humulus
  - Joining Online Reading Communities
  - Participating in Virtual Book Clubs
  - Following Authors and Publishers Organogenesis From Internode Derived Nodules Of Humulus
9. Balancing eBooks and Physical Books Organogenesis From Internode Derived Nodules Of Humulus
  - Benefits of a Digital Library
  - Creating a Diverse Reading Collection Organogenesis From Internode Derived Nodules Of Humulus
10. Overcoming Reading Challenges
  - Dealing with Digital Eye Strain
  - Minimizing Distractions
  - Managing Screen Time
11. Cultivating a Reading Routine Organogenesis From Internode Derived Nodules Of Humulus
  - Setting Reading Goals Organogenesis From Internode Derived Nodules Of Humulus
  - Carving Out Dedicated Reading Time
12. Sourcing Reliable Information of Organogenesis From Internode Derived Nodules Of Humulus
  - Fact-Checking eBook Content of Organogenesis From Internode Derived Nodules Of Humulus
  - Distinguishing Credible Sources
13. Promoting Lifelong Learning

- Utilizing eBooks for Skill Development
  - Exploring Educational eBooks
14. Embracing eBook Trends
- Integration of Multimedia Elements
  - Interactive and Gamified eBooks

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web overview a nine day self guided walking holiday following the isle of skye trail along the length of the island the largest of the inner hebrides archipelago where lochs rugged peninsulas and valleys are wrapped around the foothills of its mountainous interior staying in locally owned guesthouses or hostels follow this long eastern trail

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web the trail with a total length of 128 km includes the finest mountain and coastal scenery like the trotternish ridge and the jagged cuillin hills coastal cliffs steep trails and the haunting ruins of deserted villages destroyed in the infamous highland clearances

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on this celebrated island and indeed in the whole of the uk the route follows much of the amazing trotternish ridge and later passes beneath the very shadow of the cuillin

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web the skye trail is a challenging unofficial long distance route through the stunning landscapes of the isle of skye the trail covers 128km of tough terrain including spectacular mountain and coastal scenery

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web the skye trail hard 4 0 106 isle of skye highlands scotland photos 37 directions print pdf map length 79 9 mielevation gain 15 265 ftroute type point to point head out on this 79 9 mile point to point trail near isle of skye highlands generally considered a challenging route it takes an average of 38 h 51 min to complete

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diese fernwanderung der skye trail in schottland ist ein inoffizieller fernwanderweg auf 129 km tourenbeschreibung mit informationen und tipps um den selbst zu wandern

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web mar 17 2020 the skye trail is an unofficial 80 mile long multi day hike taking in some of the island s best features including the old man of storr the quairaing and the cuillin range which is arguably one of the best mountain ranges in the uk this is why the trail could be considered as one of scotland s best

**the skye trail trip report backpacking guide** - May 12 2023

web oct 16 2018 at a glance distance 128 km 80 miles approx duration 6 or 7 days difficulty moderate start finish broadford south rubha hunish north which direction should i go i hiked from south to north

**hike the skye trail visitscotland** - Jun 13 2023

web the skye trail is a well known but little trodden route that stretches the length of the ancient isle of skye the 127km unmarked route takes in the island s most dramatic landscape from the trotternish ridge to the cuillin mountain range

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web le skye trail est un sentier de randonnée du royaume uni situé en Écosse sur l île de skye il se divise en sept étapes totalisant 128 kilomètres le skye trail traverse l île de skye du nord au sud en longeant sa côte orientale sur 128 kilomètres et sans marquage il débute au niveau du rubha hunish le cap septentrional de l île