INITIATION OF MICROPROPAGATION OF THE FERN – *OSMUNDA REGALIS L.*

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Background: *In vitro* micropropagation is a modern and efficient technique for plant multiplication under aseptic laboratory conditions, enabling the production of large numbers of virus-free and pathogen-free microplants. This method is independent of climatic conditions and is carried out in controlled growth chambers with regulated airflow, optimal temperature and humidity, and appropriate lighting. Biotechnological approaches are widely applied for the conservation and propagation of rare and endangered species, including ferns. The growing demand for ornamental ferns highlights the need for propagation techniques that allow rapid production of large numbers of plants.
The present study aimed to initiate and multiply *in vitro* the species *Osmunda regalis* L. within the Laboratory of Embryology and Biotechnology of "Al. Ciubotaru" National Botanical Garden (Institute).
Materials and methods: *O. regalis* L. is a perennial, deciduous fern with large, striking leaves (fronds), which grow about 150-200 cm, and are light green in summer and turn yellow-brown in autumn. The species is frost-tolerant (down to −25°C), thrives in moist, acidic soils, prefers shaded or semi-shaded sites and develops from a short, erect, massive rhizome, forming a small, trunk-like structure in mature plants. Its fronds are dimorphic: *sterile* outer fronds and *fertile* central fronds, the latter producing reddish-brown sporangia arranged in dense panicles at maturity. For *in vitro* initiation, the inoculum consisted of rhizome fragments, frond primordia and apices, incompletely differentiated sporangium fragments and immature spores, collected in early April from juvenile sporophytes. The plant material was sterilized in three stages using various sterilizing agents, while spores were packaged in filter-paper envelopes. Both sterilized plant material and spores were aseptically transferred onto nutrient media, the composition of which was tailored to specific experimental goals: spore germination, gametophyte differentiation, sporophyte development, or rhizogenesis induction. The initiated cultures were maintained in a growth chamber under 3000 lux illumination for 16 hours per day at 23 - 24°C.
Results: Following spore germination, gametophyte development begins with the formation of the prothallus, a filamentous, irregular structure differentiated into a basal chlorophyll-poor region bearing numerous trichorhizoids and unicellular brown trichomes, and several distal lobes with well-developed meristematic tissue on which antheridia and archegonia are formed. When rhizome fragments, primordia, and frond apices were used as inoculum, no positive results were observed after 30 days of incubation. However, when sporangium fragments and solitary spores were used, abundant spore germination occurred after six weeks, followed by prolific development of multicellular gametophytes.
Conclusion: The success of cultures initiated from spores depends on spore size and viability, sterilization method, culture medium, pH, temperature, light and humidity.

Keywords: *in vitro,* inocula, ferns, sporangia, spores, gametophyte.

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