



**A REVIEW-*IN-VITRO* MICROPROPAGATION OF *Matteuccia
struthiopteris* (L.) TODARO**

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ABSTRACT

In-vitro plant regeneration method developed for *Matteuccia struthiopteris* (L.) Todaro (fiddlehead fern). Micropropagation is a vegetative propagation conducted under controlled and aseptic conditions in the microenvironment of the culture vessel, which have the all growth requirements of a plant in the natural conditions. Recently different techniques of propagation have been developed which could facilitate large scale production of plants and for the improvement of the species. An overview on the *in vitro* propagation via Explant Establishment, Shoot Multiplication and Post Culture etc. collected from various review articles are presented here. Today micropropagation techniques are applied in order to produce large numbers of new high-quality plants in a relatively short time and space, in low cost and can also be preserved.

Keywords: *Matteuccia struthiopteris*: Fiddlehead Fern: Ostrich Fern: Micropropagation: Tissue Cultures

1. INTRODUCTION

Traditional medicine is sum of the total skill, practices, knowledge, beliefs, and experiences to different cultures, used in the maintenance of the health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness. Herbal medicines are the oldest remedies known to mankind. Herbs have been used by all cultures throughout history but India has one of the oldest, richest and most diverse cultural living traditions associated with the use of medicinal plants. In the present scenario, the demand for herbal products is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on plant materials for their potential medicinal value [1]. Herbal drug technology is used for converting botanical materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. Herbal formulations have reached widespread acceptability as therapeutic agents for diabetics, arthritics, liver diseases, cough and cold, and memory enhancement throughout the world. Herbals are traditionally considered harmless and increasingly being consumed by people without prescription [2].

M. struthiopteris (L.) commonly known as Fiddlehead fern, is one of the ancient traditional herb or ostrich fern, possesses fertile fronds and cataphylls in addition to vegetative leaves. All of these fronds are different from one another. In the growing season fronds are Emerge into different types is separated over. These Vegetative leaves appear in a spring flush, and the others appear a number of weeks later. This plant is natively found in cold temperate region of large stands as a dominant understorey hemi cryptophyte along floodplains in parts of the northern hemisphere in a variety of forest types [3]. Young fronds or furred vegetative fronds are harvest bases on frozen and spring vegetable industry in northeast included North America [4].

The holistic study of important changes to biodiversity by climate changes at global level and by anthropogenic impact imposed more intense concerns regarding the conservation of a significant number of endangered plant species. Ferns are one of most important groups. Propagation systems have been devised for use on a commercial scale for a number of different ferns, In time, ferns attracted worldwide numerous research teams but also in Romania in the last years due to their age, biotechnological potential

and due to a low number of ornamental studies that concern conservation by *in vitro* culture. All of which are sold as ornamentals. Growth regulating substances such as naphthalene acetic acid and kinetin have been used on *Alsophila australis*, *Microlepia strigosa*, *Adiantum cuneatum*, *Nephrolepis exaltata* var. *Bostoniensis* [5]. The Ostrich fern, *Matteuccia struthiopteris* (L.) Todaro is the commercial source of 'fiddleheads' which are sold fresh or useful frozen in the United States and Canada. In the last decade this industry has greatly expanded. There is also interest in *M. struthiopteris* as a garden ornamental in both Europe and North America. The vegetative propagation of this fern from nursery stock is very slow. *In vitro* micropropagation was undertaken to develop a more expeditious method for Ostrich fern multiplication [6]. *In*

vitro propagation of *fiddlehead fern* on solid media was achieved by Dykeman and Cumming (1985) and Hicks and von Aderkas (1986) who regenerated plantlets; the shoots from these plantlets were subsequently sub-cultured for further multiplication [7, 8].



Figure 1: Fiddlehead Fern

2. REVIEW METHODOLOGY

The following methodology was used for present review (Figure 2).

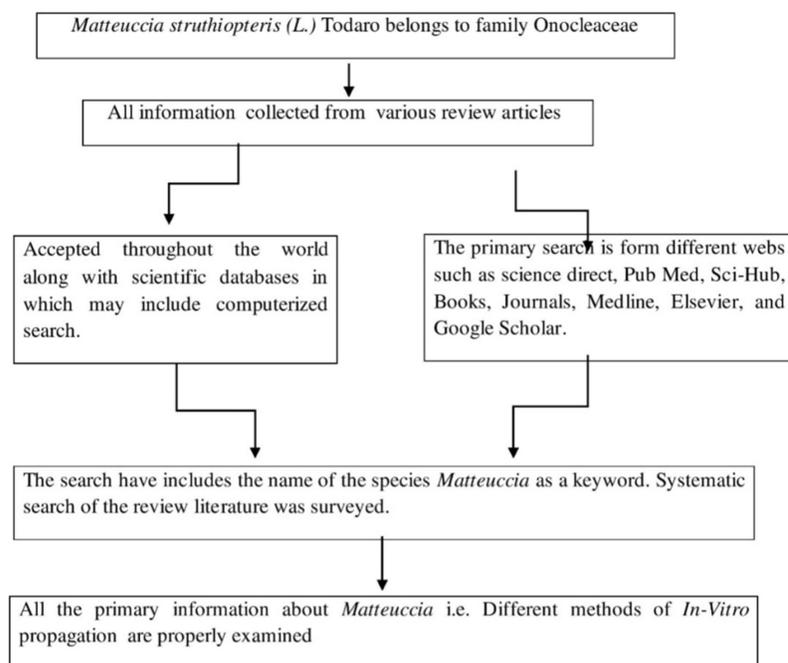


Figure 2: Flow diagram of Review methodology

4. METHODS OF TISSUE CULTURE

4.1 Explant Establishment *In-vitro* method:

Harper *et al* in 1976 developed an explant tissue culture method. They used ten shoots, each was 8 mg in weight, placed on 50 mL of medium in a 300 mL ointment Jar. Jars covered with a double layer of saran film and maintained in continuous light (1000 lx from cool white lamps). Four replications per treatment of four jars (40 shoots) each were used. After 8 wk the growth data were recorded in culture in terms of fresh weight, dry weight, number of shoots, average shoot weight, the number of fronds and roots per culture. Analysis of variance and an LSD test were carried out on all data. Afterwards three cytokinins: kinetin, N6-benzylamino purine (BA) and N6 isopentenylamino purine (2iP) and three auxins NAA, 3 indoleacetic acid (IAA) and indolebutyric acid (IBA) were initially assayed, for their effectiveness. Based on these assays, kinetin and NAA were utilized exclusively for all studies. NAA and kinetin were evaluated at concentrations of 1 0. 2.0. 4.0 and 8.0 mg/L each in a 4 x 4 factorial study. This was followed by a 5 x 4 factorial study which included NAA at concentrations of 0.0, 0.1, 0.5, 1.0 and 2.0 mg/L and kinetin at concentrations of 0. 1, 0.5, 1.0 and 2.0 mg/L.

MS inorganic macronutrient salts at half (0.5 x), three quarters (0.75 x) and full strength (1.0 X), and sucrose at concentrations of 15, 30, and 45 g/L were evaluated in a 3 x 3 factorial study. Concentrations of TC agar of 0 (using a filter bridge), 4, 8 and 12 mg/L were also evaluated [5].

In another study by Dykeman, after preliminary screening of different plant parts as source of explant, shoot produced from detached meristems on the rhizome was found suitable. So 2cm sliced rhizomes pieces were kept in plastic bags (25°C temperature and light 100- 500 lx). After 6-8 weeks when lateral shoots become 5-10 mm long, the shoot tips removed and surface sterilized. Rinsed with water twice and explant cut 1-2 mm and each placed in 30 ml ointment jar containing 15 ml of Murashige and Skoog medium [7, 11].

4.2 *In Vitro* Shoot Multiplication method

The basal medium for plantlet development studies included: MS macronutrient salts, 0.5 x: MS micronutrient salts; and, in milligrams per litre inositol, 100; thiamine.HCl, 0.4; sucrose, 7500; and TC agar, 8000. Forty shoots per treatment were used for studies. These were placed, 10 shoots to a 300 mL ointment jar with 50 mL of medium and covered by a double layer saran film. Cultures were maintained in continuous light

at 3000 lx at 25±1°C for 4 or 6 week. Plantlets were then evaluated for fresh weight, the number of fronds with three or more pinna, the number of roots greater than 5 mm in length [12].

4.3 Post Culture Establishment method

The in vitro culture to soil was best achieved by moving the culture vessels to a shaded greenhouse for 1 week prior to transplanting. Plants were then transferred to a soilless mix in 4 cm cell packs and kept in a high-humidity chamber for 2 wk. They were then moved to a regular shaded greenhouse bench. After 2 month they were transplanted to 7.5 cm peat pots.

5. CHEMICAL CONSTITUENTS

The ferns have been found to contain a high amount of phenolic compounds, glycosides, flavonoids, terpenoids, carotenoids, alkaloids, and fatty acids. Various parts are reported to have Demethoxymateucinal, Ferrol, Ferrol, matteucin, as matteucinol, methoxymatteucin, 3-hydroxy-matteucinal, cyrtominetin, 5,7-dihydroxy-4-methoxy-6-methyl-flavanone, demethoxymateucinol 7-O- glucoside etc.

6. COMMON FERNS AND THEIR USES (Table 2)

Table 2: Common fern & their uses

| Species | Country | Source and parts used | Relevant findings/Uses | References |
|---|----------------------|-----------------------|---------------------------|------------|
| Ostrich fern | Canada | Wild; youngFronds | Vegetable | [13] |
| Fiddlehead fern | Japan | Wild; swollen Rhizome | Used as vegetable | [14] |
| Bracken (<i>Pteridium aquilinum</i>), | East Asia, | Wild; wholePlant | Used as vegetable | [15] |
| Royalfern (<i>Osmunda japonica</i>) | Korea | Wild; youngFronds | Used as vegetable | [16] |
| <i>Ceratopteris thalictroides</i> (L.) | Western Ghats, India | Wild; youngFronds | Used as vegetable | [17] |
| <i>Microsorium punctatum</i> (L.) | Assam | Wild; youngFronds | Used as vegetable | [18] |
| <i>Pteridium aquilinum</i> (L.) | America | Wild; young fronds | Used for preparing Pakori | [19] |

5. CONCLUSION

Common food Uses, Taxonomy, Various photochemical & In vitro propagation studies on fiddlehead fern species presented in the study. In vitro propagation studies on fiddlehead fern species have consistently reported. A pathway for its in vitro propagation is outlined in various methods

However, these aspects of the protocol like field performance of the species, clonal integrity of the propagules and clonal specificity to in vitro propagation, are yet to be determined.

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ABBREVIATIONS

4-PU N-(4-pyridyl)-N'-phenylurea ·

BA 6-Benzylaminopurine

cm Centimeter

g/ L gram per Litre

Hcl Hydrochloric acid

indoleacetic acid (IAA).

indolebutyric acid (IBA).

Lysergic acid diethylamide (LSD).

M. struthiopteris *Matteucciaruthiopteris*.

mg Milligram

mg/ L miligram per Litre

ml Milliliters

MNs meristematic nodules ·

MS Murashige and Skoog (1962) nutrient medium ·

NAA Naphthaleneacetic acid.

TC agar Thiosulfate-citrate-bile salts-sucrose agar.

TDZ N-phenyl-N-1,2,3-thiadiazol-5- yl urea (thiadiazuron)

wk week.

x-Magnification.

REFERENCES

- [1] World Health Organization (WHO). WHO Traditional Medicine Strategy 2014-2023. *World Heal. Organ.*, **2013**, 1–76. <https://doi.org/2013>.
- [2] Kokate C.K., Purohit A. P., G. S. B. Pharmacognosy 13th Edition. **2007**, 370.
- [3] Prange, R. K.; Von Aderkas, P. Biological Flora of Canada. 6. *Matteuccia struthiopteris* (L.) Todaro, Ostrich Fern. *Canadian field-naturalist*. 1985.
- [4] Von Aderkas, P. Economic History of Ostrich Fern, *Matteuccia Struthiopteris*, the Edible Fiddlehead. *Econ. Bot.*, **1984**, 38 (1), 14–23. <https://doi.org/10.1007/BF02904412>
- [5] Harper, K. L. Asexual Multiplication of Leptosporangiate Ferns through Tissue Culture. University of California, Riverside 1976.
- [6] Murashige, T. Plant Propagation Through Tissue Cultures. *Annu. Rev. Plant Physiol.*, **1974**, 25 (1), 135–166. <https://doi.org/10.1146/annurev.pp.2>

- 5.060174.001031.
- [7] Dykeman, B. W. Effects Of Crozier Removal On Growth Of The Ostrich Fern. *Can. J. Plant Sci.*, **1985**, *65* (4), 1019–1023.
<https://doi.org/10.4141/cjps85-130>.
- [8] Hicks, G.; Von Aderkas, P. A Tissue Culture of the Ostrich Fern *Matteuccia Struthiopteris* (L.) Todaro. *Plant Cell. Tissue Organ Cult.*, **1986**, *5* (3), 199–204.
<https://doi.org/10.1007/BF00040130>
- [9] Kimura, T.; Suzuki, M.; Takenaka, M.; Yamagishi, K.; Shinmoto, H. L-O-Caffeoylhomoserine from *Matteuccia Struthiopteris*. *Phytochemistry*, **2004**, *65* (4), 423–426.
<https://doi.org/https://doi.org/10.1016/j.phytochem.2003.10.008>.
- [10] Kimura, T.; Yamagishi, K.; Suzuki, M.; Shinmoto, H. Relative Estimation of the Radical Scavenging Activities of Agricultural Products. *J. Food Sci. Technol.*, **2002**, *49* (4), 257.
<https://doi.org/10.3136/nskkk.49.257>.
- [11] Murashige, T.; Skoog, F. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiol. Plant.*, **1962**, *15* (3), 473–497.
<https://doi.org/https://doi.org/10.1111/1/j.1399-3054.1962.tb08052.x>.
- [12] Cheng, T. Y.; Voqui, T. H. Regeneration of Douglas Fir Plantlets through Tissue Culture. *Science*, **1977**, *198* (4314), 306–307.
<https://doi.org/10.1126/science.198.4314.306>.
- [13] Delong, J.; Hodges, D.; Prange, R.; Forney, C.; Toivenon, P.; Bishop, M. C.; Elliot, M.; Jordan, M.; Delong, J.; Hodges, D.; et al. The Unique Fatty Acid and Antioxidant Composition of Ostrich Fern (*Matteuccia Struthiopteris*) Fiddleheads. *Can. J. Plant Sci.*, **2011**, *91*, 919.
<https://doi.org/10.4141/CJPS2010-042>.
- [14] Ikeya, K. Cultural Ecology of Zenmai Gathering in the Northeastern Japan. *Sci. Reports Tohoku Univ. Ser. 7 Geogr.*, **2004**, *53*.
- [15] Hodge, W. H. Fern Foods of Japan and the Problem of Toxicity. *Am. Fern J.*, **1973**, *63* (3), 77–80.
<https://doi.org/10.2307/1546182>.
- [16] Pemberton, R. W.; Lee, N. S. Wild Food Plants in South Korea; Market

- Presence, New Crops, and Exports to the United States. *Econ. Bot.*, **1996**, 50 (1), 57–70.
<https://doi.org/10.1007/BF02862113>
- [17] Karthik, V.; Raju, K.; Ayyanar, M.; Gowrishankar, K.; Sekar, T. Ethnomedicinal Uses of Pteridophytes in Kolli Hills, Eastern Ghats of Tamil Nadu, India. *J. Nat. Prod. Plant Resour*, **2011**, 1 (2), 50–55.
- [18] Sharma, U.; Pegu, S. Ethnobotany of Religious and Supernatural Beliefs of the Mising Tribes of Assam with Special Reference to the “Dobur Uie.” *J. Ethnobiol. Ethnomed.*, **2011**, 7, 16.
<https://doi.org/10.1186/1746-4269-7-16>.
- [19] Foster, S.; Duke, J. *Peterson Field Guide to Medicinal Plants and Herbs: Eastern and Central North America.*; 2014.