Review Article

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IN VITRO PROPAGATION IN PTERIDOPHYTES: A REVIEW

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ABSTRACT

Pteridophytes, by virtue of possessing great variety and fascinating foliage, have drawn the attention and admiration of horticulturists and plant lovers for centuries. Characterized by an alternation of generations between a well developed sporophyte producing spores and an independent gametophyte producing gametes, the Pteridophyta include over 12,000 species, many of which are ornamental species, medicinal species, species of an ethnobotanical importance with a role in habitat conservation etc. Medicinal value of pteridophytes is known to man for more than 2000 years. The rare and economically important pteridophyte species should be treated as our natural heritage and properly conserved. *In vitro* propagation is the alternative method for propagation and conserving these species.

Keywords: *In vitro* propagation, pteridophytes, sporophyte, gametophyte, plant growth regulators.

INTRODUCTION

The pteridophytes include the fern and fern allies and they are the vascular plants that produce spores rather than seeds. The spores are produced in sporangia borne in sori on the lower or abaxial surface of the leaves. The ferns usually have larger and more complex macrophyllous leaves compared to the fern allies. The fern allies have sporangia associated with sporophylls and the leaves are microphyllous¹. The pteridophytes constitute a significant part of the earth's plant diversity and being the second largest group of vascular plants, they form a dominant component of many plant communities. Most of the ferns grow as thick ground cover and hence provide a good means to prevent soil erosion and they invade easily into exposed areas.

The pteridophytes are dependent upon the microclimatic conditions of the region for their successful survival in that region. Any kind of disturbance can hinder the evolutionary process leading to their population decline. Factors such as climatic change, industrialization, encroachment of forest lands, over exploitation of natural resources, large scale collection of ferns from the forests by visitors and local people for ornamental purpose, medicinal purpose and during excursions, etc. pose a major threat to the survival of these groups of plants.

Pteridophyta are represented by about 305 genera, comprising more than 10,000 species all over the world. About 191 genera and more than 1000 species are reported from India. It has become imperative to develop *in situ* and *ex situ* conservation methods for conservation of the diminishing biodiversity. *Ex situ* conservation for the endemic and rare pteridophytes species can be suggested by collecting live specimens from the wild and grow them in pots for propagation in Fernery². *In vitro*

culture technology can also be used as the alternative method for propagation and conserving these species.

The concept of plant tissue culture was first envisioned by Haberlandt³. His pioneering research work provided the groundwork for the first instances of callus induction and sustainable cell growth nearly forty years after his original experiments. The culture of tissue in ferns has been utilized as research instrument for the study of the developing potentialities of the leaf primordia ever since the early 1960's⁴.

The first successes in the field of the intensive multiplication of plants through *in vitro* techniques are cited around 1970, the fern *Nephrolepis exaltata bostoniensis* being the first plant micropropagated *in vitro* with a commercial purpose⁵. 157 million plants, i.e. 74% out of the total production of micropropagated plants, have been ornamental species⁶. Out of these, approximately 40 million plants have been pot plants. Top of the list, with 17.8 million plants, is the fern *Nephrolepis*⁷.

CONCLUSION

Of all the ferns studied from 1925 to 2012 for *in vitro* propagation, some are of medicinal value such as *Ampeleopteris prolifera*, *Drymoglossum piloselloides*, *Pyrrosia piloselloides*, *Marsilea quadrifolia*, *Osmunda regalis*, *Dryopteris filix-mas*, *Platycerium coronarium*, *Helminthostachys zeylanica*, *Bolbitis costata*, *Drynaria fortunei*, *Asplenium nidus*, *Adiantum sp.*, *Ceterach officinarum*, *Pityrogramma calomelanos*, *Adiantum capillus veneris*, *Lygodium japonicum*, *Blechnum spicant*, *Pteris ensiformis*, *Equisetum arvense*, *Drynaria quercifolia*, *Pteridium aquilium*, *Osmunda japonica*, *Dicksonia sellowiana*, *Pteris multifida*, *Sphaeropteris hainanensis*, *Huperzia selago*, *Cystopteris fragilis*,

Table 1: Tabulation showing trend of work done for *in vitro* propagation in pteridophytes from 1925 to 2012

Species	Family	Explant	Medium	Observation	Ref
<i>Onoclea sensibilis</i>	Dryopteridaceae	Spores	Double distilled water & agar	Germinate best in intense diffused light at 28°C; absolute darkness at 28-29°C.	8
<i>Pteridium aquilinum</i> var <i>latisculum</i>	Pteridaceae	Spores	Knop's, Knudson's Solution	Callus growth in Knop's solution with 2% glucose & 0.5g/L yeast extract.	9
<i>Ampelopteris proliferata</i>	Thelypteridaceae	Rhizome	Mineral nutrients with sucrose	Sucrose (5-8%) detrimental to prothallial growth & apogamy delayed with 2-3% sucrose; Callus induction in 2,4-D (3-5mg/L).	10
Several ferns including maiden hair fern (<i>Adiantum</i> sp.)	Adiantaceae	Spores	½ MS medium	Gametophytic stage observed in <i>in vitro</i> culture & sporophyte formation when comminuted in a blender for 5 sec in ½ MS.	11
<i>Nephrolepis exaltata</i> var <i>bostoniensis</i>	Oleandraceae	Runner tissues	MS medium	Organogenesis with dilute solution of standard MS medium; Shoot development promoted by NAA and KIN & root initiation by 2,4-D and KIN.	12
<i>Cheilanthes microphylla</i>	Pteridaceae	Spores	Hogland's No.2 solution	Sporophyte emerge from gametophytes.	13
<i>Nephrolepis</i> sp.	Oleandraceae	Young stolons	Gamborg B ₅ medium	Leaf primordial production with IBA (1mg/L); White friable callus with 2,4-D (2mg/L) at the cut ends of stolon segments.	14
<i>Rumohra adiantiformis</i>	Dryopteridaceae	Rhizome tips	Modified Prague's medium	Large scale micropropagation with 2-iP (1.0mg/L), KIN (1.0-1.5mg/L) or Zeatin (1mg/L).	15
<i>Nephrolepis falcata</i>	Oleandraceae	Shoots	Sterile tissue culture medium	Maximal shoot production with KIN (5X10 ⁻⁷ & 10 ⁻⁶ M) & no NAA.	16
<i>Matteuccia struthiopteris</i>	Dryopteridaceae	Shoots produced from detached meristems on the rhizome	Solid MS medium	Maximal shoot proliferation with KIN (1.0mg/L) in ½ MS.	17
<i>Matteuccia struthiopteris</i>	Dryopteridaceae	Shoot tips	Semisolid Knudson's medium	Multiple bud formation with KIN (10 ⁻⁵ & 10 ⁻⁶ M)	18
<i>Pteridium aquilinum</i> , <i>Cyclosorus contiguus</i> , <i>Dryopteris filixmas</i> , <i>Pteris henryi</i> , <i>Osmunda regalis</i> , <i>Pteris cretica</i> vs. <i>albolineata</i> , <i>Marsilea quadrifolia</i> , <i>Pilularia globulifera</i>	Pteridaceae Thelypteridaceae Dryopteridaceae Pteridaceae Osmundaceae Pteridaceae Marsileaceae Marsileaceae	Spores, excised shoots	MS, Moore's medium	Apogamous sporophyte organization with Auxins (IAA, NAA), Cytokinins (KIN, BA) and 0.5% saccharose. Sporophytes maintained in ½ MS hormone free.	19
<i>Cyrtomium falcatum</i>	Dryopteridaceae	Spores	MS medium	Fast growing tissue with KIN (2mg/L) & NAA (0.1mg/L) or BAP (3mg/L) & NAA (0.1mg/L) and better leaf elongation with GA ₃ (0.1mg/L).	20
<i>Nephrolepis cordifolia</i>	Oleandraceae	Spores	MS medium	Micropropagation was reported through the proliferation of green globular bodies (GGBs) with cytokinin; these GGBs later developed into sporophytes.	21
<i>Adiantum capillus veneris</i>	Adiantaceae	Circinate portion of young foliar leaves	Gamborg B ₅ medium	Best morphogenic response with IBA (0.5mg/L), BA (0.01mg/L) and 2% sucrose at pH 5.5 & rooting with NAA (0.05mg/L).	22
<i>Drymoglossum piloselloides</i>	Polypodiaceae	Fronds	Modified MS medium	Apogamous gametophytes production with KIN; NAA enhanced the effect of KIN.	23
<i>Lygodium japonicum</i>	Lygodiaceae	Protoplasts from juvenile leaflets	MS medium	Cell clusters (10-15 cells) with NAA (2.7µM), BAP (2.2µM), 0.6 M mannitol & 0.05 M sucrose; Rhizoids and protenema-like regenerants in hormone free medium containing no mannitol.	24
<i>Botrychium dissectum</i>	Ophioglossaceae	Spores	Media without N ₂	Reduced N ₂ source (0.035mol m ⁻³) necessary for spore germination & early growth of gametophytes.	25
<i>Pyrrosia piloselloides</i>	Polypodiaceae	Spores, frond strips	Modified MS medium	Aposporous gametophytes with sugar alcohols (sorbitol, mannitol), auxins (NAA, 2,4-D) & Cytokinin (BA) in 1% agar.	26
<i>Nephrolepis</i> , <i>Asplenium</i> , <i>Pteris</i> , <i>Adiantum</i> , <i>Rumohra</i>	Oleandraceae Aspleniaceae Pteridaceae Adiantaceae Dryopteridaceae	Spores	Modified MS medium	Green globular bodies(GGB) proliferated in BA; Plantlet regenerated in BA free medium.	27
<i>Pteris vittata</i>	Pteridaceae	Pinnae strips	Modified MS medium	Aposporous gametophytes and callus production.	28
<i>Platyserium bifurcatum</i>	Polypodiaceae	Spores	MS medium	Gametophytes production at higher concentration of sugar alcohols, growth regulators and sugars. Sporophyte regeneration in hormone free MS from callus & in 1/10 MS from aposporous gametophytes.	29
<i>Platyserium bifurcatum</i>	Polypodiaceae	<i>In vitro</i> grown leaves	MS medium	Adventitious bud development with 6-BA (0, 5 or 10µM) and rooting in IBA (6µM).	30

<i>Ceratopteris thalictroides</i>	Parkeriaceae	Adventitious bud & leaf	MS medium	Multiple shoots induced from adventitious buds and leaf callus.	31
<i>Salvania natans</i>	Salviniaceae	Spores	MS medium	Protoplasts culture from young Sporophytes.	32
<i>Platyserium coronarium</i>	Polypodiaceae	Spores	MS medium	Highest percentage of apogamous sporophytes per gametophyte clump with 40µM IAA.	33
<i>Blechnum spicant</i> <i>Pteris ensiformis</i>	Blechnaceae Pteridaceae	Rhizomes	MS medium	Several proliferation centres observed with BA (0.44-4.4µM) alone or in combination with NAA (0.053-0.53µM).	34
<i>Platyserium bifurcatum</i>	Polypodiaceae	Shoots obtained <i>in vitro</i> from spores	MS medium	Neutral or acid pH stimulates <i>in vitro</i> frond production. Optimal shoot multiplication with NAA (0.54µM) & KIN (9.3µM) at pH 7.	35
<i>Blechnum spicant</i>	Blechnaceae	Spore	MS liquid medium	IBA (5µM & 50µM) & BA (50µM) inhibited antheridiogen activity; GA ₃ allowed spore germination but inhibited gametophyte development.	36
<i>Asplenium nidus</i> <i>Pteris ensiformis</i>	Aspleniaceae Pteridaceae	Rhizomes	MS medium	Homogenization of rhizomes pretreated with 4.4µM 6-BA developed sporophytes on hormone free MS.	37
<i>Osmunda regalis</i>	Osmundaceae	Gametophytes	Knop's, Knudson's, ¼ MS medium	Gemmae formation with sucrose in the medium & in light.	38
<i>Platyserium grande</i> <i>Asplenium nidus</i> <i>Blechnum orientale</i> <i>Cyathea contaminans</i> <i>Leucostegia immersa</i> <i>Asplenium decorum</i> <i>Pteris mutilata</i> <i>Adiantum capillus veneris</i> <i>Pteris mutilata</i> var <i>victoriae</i> <i>Osmunda banksiifolia</i> <i>Lygodium japonicum</i> <i>Lygodium flexuosum</i>	Polypodiaceae Aspleniaceae Blechnaceae Cyatheaceae Davalliaceae Aspleniaceae Pteridaceae Adiantaceae Pteridaceae Osmundaceae Lygodiaceae Lygodiaceae	Spores	MS medium	Prothalli formation which later gave rise to sporophytes.	39
<i>Matteuccia struthiopteris</i>	Dryopteridaceae	Side shoots from meristems of sectioned rhizome	½ MS liquid medium	Meristem multiplication with 4-PU (2.0mg/L) and TDZ (0.5mg/L); Rhizogenesis in hormone free ¼ MS solid with 0.4% agar & 1.0% activated charcoal.	40
<i>Polypodium cambricum</i>	Polypodiaceae	Rhizome, frond, petiole or root tip	MS liquid & modified medium	Green globular bodies (GGB) in hormone free medium; Homogenization of BA pretreated frond or rhizomes improved fern propagation.	41
<i>Dicksonia sellowiana</i>	Dicksoniaceae	Spores	100 units nystatin per mL liquid medium	White light & 731 days of cold storage (10 ⁰ C) favoured 82% spore germination.	42
<i>Woodwardia virginica</i> <i>Dryopteris affinis</i> <i>Osmunda regalis</i> <i>Pteris ensiformis</i>	Blechnaceae Dryopteridaceae Osmundaceae Pteridaceae	Spores	½ MS basal medium	Homogenization of gametophytes & 0.7% agar and water medium yielded higher sporophytes; MS basal without sucrose favoured leaf expansion.	43
<i>Equisetum arvense</i>	Equisetaceae	Spores	MS liquid medium	Globular cell mass with cytokinin; Normal young gametophytes without cytokinin	44
<i>Lycopodiella inundata</i>	Lycopod	Vegetative apices	Few minerals & organic compound medium	Nodular calluses with IBA(0.05µM) & KIN(1.4µM); Plantlet regeneration with IBA(2.5µM) & GA ₃ (0.33µM).	45
<i>Drynaria quercifolia</i>	Drynariaceae	Rhizome	MS medium	Sporophyte production from rhizome culture	46
<i>Osmunda regalis</i>	Osmundaceae	Spores	Hoagland & Arnon	Sporophytes looked normal and showed considerable growth on hormone free growth medium. Tissue vitrification when cytokinins used.	47
<i>Cyathea contaminans</i>	Cyatheaceae	Spores	MS medium	Antheridial development from isolated protoplast of young gametophytes	48
<i>Pteridium aquilium</i>	Dennstaedtiaceae	Spores	MS medium	Spores germinate after 4-5 days, gametophytes in 20 days & sporophyte after fertilization in 10 days.	49
<i>Platyserium bifurcatum</i>	Polypodiaceae	Leaf	Modified MS medium	Low sugar concentration (0.01%) and wounding of leaf adaxial side induced 90% of leaves to produce aposporous gametophytes.	50
<i>Pteridium apuilinum</i>	Dennstaedtiaceae	Different explants	½ MS medium	Plant regeneration from callus with BA(0.2mg/L), IBA(0.4mg/L) & NH ₄ N ₂ PO ₄ (200mg/L).	51
<i>Osmunda japonica</i>	Osmundaceae	Spores	MS medium	3g spore powder per square metre produce 3000 sporophytes by spraying 2,4-D, GA ₃ (50mg/L) & 1% KH ₂ PO ₄ .	52
<i>Nephrolepis biserrata</i>	Nephrolepidaceae	Fronds from stolon segment culture	MS semi-solid medium	Sucrose (15-45g/L) increased leaf length and diameter at pH 5 to 7.	53
<i>Osmunda cinnamomea</i>	Osmundaceae	Spores	Membrane filter sheet laid on moist Sphagnum moss	Gametophyte size negatively related to population density, which significantly affects gametophytes' sexual expression.	54
<i>Pteris multifida</i>	Pteridaceae	Spores	MS medium	Spores germinate within 7-9 days, the germ filament develop 3 cells long, prothallus matured in 80-100 days after inoculum.	55

<i>Cyathea lepifera</i>	Cyatheaceae	Spores	½ MS without sugar	Sporophytes developed from the <i>in vitro</i> cultured gametophytes.	56
<i>Dicksonia sellowiana</i>	Dicksoniaceae	Spores	Dyer & MS medium	Lower spore germination with sucrose & 5% and 20% of irradiance. Dry mass of 30 day old gametophyte was higher in Dyer medium (3-5% sucrose) & decreased in MS (4-5% sucrose)	57
<i>Marsilea minuta</i> & <i>Ceraptoteris pteroides</i>	Marsileaceae Marsileaceae	Spores	Knop's, Knudson, Moore, MS medium	Multiple bud formation & plant regeneration with Knop's medium.	58
<i>Cyathea spinulosa</i>	Cyatheaceae	Spores	Parker & Thompson media	Fertilization success in parent and regenerated gametophytes was dependent on water availability at the time of maturity of both the sexes.	59
<i>Pteris vittata</i>	Pteridaceae	Leaf primordium segments	MS medium	Callus induction with 2,4-D (2mg/L) & BAP (0.5mg/L).	60
<i>Huperzia selago</i>	Huperziaceae	Shoots	½ MS medium	Highest growth frequency & somatic embryogenesis with ½ MS.	61
<i>Athyrium yokoscense</i>	Athyriaceae	Spores	Liquid culture	Cadmium tolerance was independent of the plant parts, although roots have higher accumulation ability.	62
<i>Platynerium bifurcatum</i>	Polypodiaceae	Spores	MS medium	Rhizoid elongation with jasmonic acid (0.01-1µM). Cell division inhibited if JA exceeds 1µM.	63
<i>Drynaria quercifolia</i>	Drynariaceae	Leafy structures, Rhizome tips	Knop's medium	Callus induction in 2,4,5-T (5mg/L), 20g/L sucrose, 8 g/L agar; leafy structure as sporophyte initials in 2-iP (10mg/L).	64
<i>Pityrogramma calomelanos</i>	Hemionitidaceae	Crozier & frond explants	½ MS medium	Aposporous gametophytes with BA(3.33µM) & KIN(2.32µM); Sporophytes developed in 1/10 MS without sucrose.	65
<i>Dryopteris affinis</i>	Dryopteridaceae	Spores	MS solid medium	Apogamous sporophyte formation with NAA (0.53 & 5.37µM) or GA ₃ (2.8µM).	66
<i>Blechnum spicant</i>	Blechnaceae	Spores	MS liquid medium	BA influenced sexual organ formation (maleness) but does not change the female sexual pattern in spore derived gametophytes.	67
<i>Blechnum spicant</i>	Blechnaceae	Spores, homogenized mature gametophytes	MS medium	GA ₄₊₇ had slight effect of inducing antheridia or archegonia; Flurprimidol did not inhibit antheridiogen biosynthesis or antheridia formation.	68
<i>Sphaeropteris hainanensis</i>	Cyatheaceae	Sporangiums (spores)	½ MS medium	Prothallium multiplication with BA (2mg/L), NAA (0.1mg/L) & 5g/L agar.	69
<i>Drynaria fortunei</i>	Polypodiaceae	Spores	½ MS medium	Highest germination in 2% sucrose at pH 7.7 under white light condition.	70
<i>Blechnum</i> sp. <i>Cibotium</i> sp. <i>Cyathea</i> sp. <i>Dicksonia</i> sp.	Blechnaceae Dicksoniaceae Cyatheaceae Dicksoniaceae	Spores	MS medium	Sucrose in the medium stimulated gametophytic multiplication but its lack induced gemmae formation.	71
<i>Asplenium nidus</i>	Aspleniaceae	Spores	½ MS basal medium	Maximum number of shoots with BAP (0.5mg/L) & NAA (2mg/L); Rooting in IBA (2mg/L).	72
<i>Phlegmariurus squarrosus</i>	Huperziaceae	Spores	Gamborg's B ₅ medium	<i>In vitro</i> propagated tissues produce higher levels of Huperzine A than the natural plant.	73
<i>Cystopteris fragilis</i> <i>Cyrtomium falcatum</i> <i>Phegopteris connectilis</i>	Dryopteridaceae Dryopteridaceae Thelypteridaceae	Green sporangia	Knop liquid medium	Amphimixis or apomixis result in sporophyte formation	74
<i>Pteris vittata</i>	Pteridaceae	Spores	½ MS medium	Callus formation in BA (0.5mg/L) + GA ₃ (0.5mg/L); Sporophyte formation with GA ₃ (1.0mg/L) + BA (0.5mg/L) + LH (300mg/L).	75
<i>Marsilea quadrifolia</i>	Marsileaceae	Leaf mesophyll cells, Nodal area of rhizomes	Knop's, ½ MS medium	Polymorphic aspect of the chloroplast in <i>in vitro</i> culture represent adaptive modifications to culture conditions	76
<i>Drynaria roosii</i>	Polypodiaceae	Spore	Knop's medium, ½ MS medium	MS basal medium favoured spore germination but inhibited gametophyte development.	77
<i>Bolbitis costata</i>	Bolbitidaceae	Spores	MS medium	Spore germination in IAA (0.2mg/L); gametophytic growth in IAA (0.4mg/L); sporophytic growth in IAA (4mg/L) + KIN (5mg/L) + IBA (0.2mg/L).	78
<i>Helminthostachys zeylanica</i>	Helminthostachya ceae	Spores	MS medium, Parker-Thompson Basic C	Maximum germination in Parker & Thompson medium with IAA (0.2mg/L) & sucrose (0mg/L).	79
<i>Dryopteris affinis</i>	Dryopteridaceae	Green sporangia	½ MS medium	Embryo formation without hormones & first root formed when sporophyte had 2-3 leaves.	80
<i>Adiantum capillus veneris</i> <i>Asplenium adiantum nigrum</i> <i>Ceterach officinarum</i> <i>Davallia canariensis</i> <i>Dicksonia antarctica</i>	Adiantaceae Aspleniaceae Aspleniaceae Davalliaceae Dicksoniaceae	Rhizomes	MS basal medium	Numerous fronds developed when rhizome pretreated with BAP. Aposporous gametophyte formation in BAP (4.4 1M).	81

<i>Dryopteris dilatata</i> <i>Dryopteris filix-mas</i> <i>Polypodium cambricum</i>	Dryopteridaceae Dryopteridaceae Polypodiaceae				
<i>Cibotium barometz</i>	Dicksoniaceae	Spores	MS medium	Sporophyte induction without hormones in 1/10 MS medium; Sporophyte multiplication in ¼ MS + KIN(2mg/L) + NAA(0.1mg/L) + 3g/L sucrose; Rooting in 1g/L active carbon.	82
<i>Platycerium bifurcatum</i>	Polypodiaceae	Juvenile leaf	MS medium	Green globular bodies (GGBs) with NAA (5.37µM) and 2-ip (2.22µM). Multiple sporophytes from GGBs on a hormone-free MS medium.	83
<i>Pronephrium triphyllum</i> <i>Sphaerostephanos unitus</i>	Thelypteridaceae Thelypteridaceae	Spores	Knop's medium	For <i>P. triphyllum</i> , the highest percentage of spore germination (38.3±1.13) was on Knop's basal agar & sporophyte formation (52.3±1.43) in Knop's liquid medium & that of <i>S. unitus</i> , germination in Knop's basal agar (36.8±1.31) and sporophyte formation (76.8±1.41) in Knop's medium.	84
<i>Drynaria quercifolia</i>	Polypodiaceae	Spores	MS, Parker & Thompson medium	Spore germination on MS+1mg/L IAA + 5mg/L KIN + 20% CM + 300mg/L CH; Sporophyte & rhizome proliferation with 5mg/L IAA and 2mg/L KIN.	85
<i>Pteris vittata</i>	Pteridaceae	Leaf primordium	MS, Parker & Thompson medium	80% callusing on full strength MS with 2,4-D (2.26µM) and BAP (2.22µM). More shoot differentiation (2.8±0.06) from calli on ¼ Parker and Thompson medium with BAP (4.44µM) and NAA (2.68µM).	86
<i>Cyathea spinulosa</i>	Cyatheaceae	Leaf primordium	Parker & Thompson	Callus induction with 8.87µM BAP and 2.21µM 2,4-D; multiple shoots with 4.52µM BAP and 5.36µM NAA & rooting with 2.24µM IBA.	87
<i>Anemia rotundifolia</i>	Schizaeaceae	Spores	Parker & Thompson	Secondary regenerates gave rise to numerous tertiary gametophytes that produce multiple sporophytes.	88

Abbreviations

MS- Murashige and Skoog; NAA- α -Naphthalene acetic acid; IAA- Indole-3-acetic acid; IBA- Indole-3-butyric acid; KIN- Kinetin; GA₃-Gibberellic acid; BA- 6-benzyladenine; BAP- Benzylaminopurine; 2,4-D- 2,4-dichlorophenoxyacetic acid; 2,4,5-T - 2,4,5-trichlorophenoxyacetic acid; JA-Jasmonic acid; GGBs- Green globular bodies.

Marchantia polymorpha, *Cyrtomium falcatum*, *Ceterach officinarum*, *Cibotium barometz*; whereas edible ferns are *Matteuccia struthiopteris*, *Pteris ensiformis* (beverage), *Asplenium* (sprouts as vegetable), *Ampelopteris prolifera*, *Pyrrosia piloselloides* (Chinese herbs), *Helminthostachys zeylanica* (vegetable), *Pteridium aquilinum* var *latisculum* (edible fiddle heads), *Ceratopteris thalictroides*, *Equisetum arvense*, *Pteridium aquilum*, *Nephrolepis biserrata*, *Pteris multifida*, *Cystopteris fragilis* (root), *Cibotium barometz*. Ferns of ornamental purpose are *Pityrogramma calomelanos*, *Pteris ensiformis*, *Platycerium bifurcatum*, *Ampelopteris prolifera*, *Platycerium coronarium*, *Pteris cretica* vs *albolineata*, *Dryopteris filix-mas*, *Davallia canariensis*, *Matteuccia struthiopteris*, *Rumohra*, *Nephrolepis exaltata* var *bostoniensis*, *Asplenium nidus*, *Ceratopteris thalictroides*, *Salvania natans*, *Dryopteris affinis*, *Polypodium cambricum*, *Pteris ensiformis*, *Sphaeropteris hainanensis* and ferns of economic importance are *Platycerium grande*, *Asplenium nidus*, *Blechnum orientale*, *Cyathea contaminans*, *Leucostegia immersa*, *Asplenium decorum*, *Pteris mutilata*, *Adiantum capillus-veneris*, *Pteris mutilata* var. *victoriae*, *Osmunda banksiifolia*, *Lygodium japonicum*, *Lygodium flexuosum*, *Pteris vittata*.

Some ferns are endangered such as *Dicksonia sellowiana*, *Cheilanthes microphylla*, *Marsilea quadrifolia*, etc. and threatened ferns are *Pronephrium triphyllum*, *Sphaerostephanos unitus*, *Cyathea spinulosa*, *Anemia rotundifolia*, etc. *Pilularia globulifera* is nearly threatened in IUCN Red List. *Rumohra adiantiformis* is facing human induced threats. *Salvania natans* is declining in European range. *Helminthostachys zeylanica*, *Osmunda cinnamomea* and *Cibotium barometz* are at risk.

Sphaeropteris hainanensis almost lost. *Osmunda cinnamomea* provide living area for many small animals, birds and many insects and hence valuable to the ecosystem is also at risk.

Though lots of works for conservation of medicinal, economically important, edible and ornamental ferns had been done, a review of all these works suggests that no or very less conservation work for rare and medicinal ferns such as *Dipteris wallichii* had been carried out till date. *In vitro* propagation work of *D. wallichii* and such other ferns could be undertaken to avoid its expected extinction in near future. The paper thus reviewed over all methods, use of plant growth regulators, plant tissue culture medium and explants used for *in vitro* propagation of these ferns and classified the works done based on economic utility of the plants. The tissue culture i.e. *in vitro* propagation is a very useful and important technique for the mass multiplication of the desired plant species in a short time and hence, research work done for developing a protocol for *in vitro* regeneration of ferns and fern allies should be appreciated.

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