SUMMARY

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In the present experimentations, detailed in vitro morphogenetic studies were carried out using two distinct ferns viz; Marsilea minuta (an aquatic heterosporous fern) and Ceratopteris thalictroides (an aquatic homosporous fern) in order to investigate different aspects of morphogenesis i.e. in vitro regeneration of rhizome segments (with and without node) of Marsilea minuta; spore germination in fern Ceratopteris thalictroides; induction of callus from different plant parts of both the ferns and development of adventitious buds from leaf explants (both intact and excised) of fern Ceratopteris thalictroides.

To study the regenerative potentialities of rhizome segments (with or without node) of Marsilea minuta, these were cultured on Knop's medium solidified with 8 gm/l agar. Rhizome segments with node produced a green bud like structure after 3-4 days which further regenerated an adult sporophyte bearing quadrifoliate leaves. It was observed that rhizome segments without node failed to regenerate and remained quiescent throughout and ultimately died.

Callus was induced from different parts of sporophytes of fern Marsilea minuta (i.e. rhizome segments with node, leaves and roots) on basal Murashige and Skoog's medium with different growth regulators including both auxins and

cytokinins. Rhizome segments (with node) induced callus on basal MS medium with 2,4-D (0.5 mg/l to 2 mg/l); NAA (0.5 mg/l to 2 mg/l); Kinetin (0.5 mg/l to 4 mg/l) and BAP (0.5 mg/l to 4 mg/l). Optimal callus was observed on Kinetin (0.5 mg/l to 2 mg/l). Maximum shoot differentiation was observed from the rhizome with node callus obtained on NAA (0.5 mg/l). Rhizome callus induced on basal MS medium with 2,4-D (0.5 mg/l to 2 mg/l) was mainly rhizogenic.

Callus was initiated from leaf explants of Marsilea minuta (basal MS + 0.5 mg/l to 3 mg/l; 0.5 mg/l to 1 mg/l NAA; 0.5 mg/l Kinetin and 0.5 mg/l BAP). Profuse leaf callus was obtained on 2,4-D (0.5 mg/l to 3 mg/l). On furtner subculturing it differentiated roots in abundance. Leaf callus induced on 0.5 mg/l BAP differentiated shoots with quadrifoliate leaves.

Root callus was obtained on basal MS medium containing 0.5 mg/l to 1 mg/l 2,4-D; 0.5 mg/l NAA; 0.5 mg/l Kinetin and 0.5 mg/l BAP). Callus induced on 1 mg/l 2,4-D differentiated few roots on subsequent culturing but no shoot differentiation was ever observed.

In order to study the spore germination of Ceratopteris thalictroides these were inoculated on Knop's medium with 8 gm/l agar. Germination of spores was observed after 5-6 days of culturing. Newly formed germ tube divided by an unequal

transverse division forming a large protonemal cell initial and small rhizoidal cell initial. Protonemal cell initial under went successive transverse divisions forming the filamentous protonema and differentiated chloroplast. On further subculturing, filamentous protonema divided by repeated transverse and longitudinal divisions and formed spatulate prothalli. Rhizoidal cell initial developed a hyaline thread like rhizoid. In the present experiment no apical cell was observed. Further growth of the spatulate prothalli was affected by a group of meristematic cells arranged in a row. Number of rhizoids was also increased. Three types of gametophytes were obtained i.e., I spatulate, II cordate symmetrical and III cordate asymmetrical. Development of sex organs was observed after 5 weeks. Antheridia developed first (i.e. after 5 weeks), while archegonia developed later (i.e. after 7 weeks). Spatulate prothalli always bore antheridia but never bore archegonia.

Gametophytic callus was induced on both tne media i.e. Knop's + 0.5% S and basal MS containing various growth regulators (i.e. 2,4-D; NAA; Kinetin; and BAP, alone). It was observed that callus obtained on Knop's + 0.5% S medium with growth regulators (0.5 mg/l to 3 mg/l 2,4-D; 0.5 mg/l NAA; 0.5 mg/l Kinetin) was slow growing and differentiated few shoots. Gametophytic callus induced on basal MS medium containing growth regulators (0.5 mg/l to 5 mg/l 2,4-D;

0.5 mg/l to 4 mg/l NAA; 0.5 mg/l to 6 mg/l Kinetin and 0.5 mg/l to 4 mg/l BAP) was healthy and proliferating. Solid, nodular, and heterogeneous callus was induced from gametophytes on basal MS + 2,4-D (0.5 mg/l to 5 mg/l). Callus induced on 0.5 mg/l 2,4-D differentiated roots on transference to basal MS medium. Differentiation of shoot in abundance was observed from the gametophytic callus induced on Kinetin (0.5 mg/l to 3 mg/l). Shoots differentiated from the gametophytic callus on 0.5 mg/l Kinetin when transferred on basal MS medium regenerated complete sporophytes and on further subculturing on basal MS medium produced adventitious buds on their leaf margin.

Callus was induced from leaf explants on basal MS medium with 2,4-D (0.5 mg/l to 5 mg/l) and NAA (0.5mg/l to 3 mg/l); Kinetin (0,5 mg/l to 6 mg/l) ; and BAP (0.5 mg/l to 7 mg/l). Optimal callus growth was observed on Kinetin (0.5 mg/l to 2 mg/l); and BAP (0.5 mg/l to 3 mg/l). Leaf callus induced on 0.5 mg/l Kinetin; and 1 mg/l BAP differentiated shoots in abundance.

Root explants inoculated on MS medium differentiated aposporous gametophytes after 10 weeks of culturing. These newly formed gametophytes were slow growing and smaller than normal ones. They did not differentiate sex organs even on prolonged culturing on the same medium. Root callus was induced on basal MS medium containing growth regulators

(0.5 mg/l to 1 mg/l 2,4-D ; 2 mg/l and 4 mg/l NAA; 0.5 mg/l Kinetin). On further subculturing, calli turned brown and failed to differentiate except root callus obtained on 0.5 mg/l 2,4-D differentiated isolated root after 8 weeks.

Leaf explants (both intact and excised) when inoculated on both Knop's + 0.5% S and basal MS medium developed buds on the leaf margin and notch of the leaf explants. It was observed that maximum number of buds were differentiated from the leaf explants intact with parent sporophytes on basal MS medium, while optimum buds were produced from the excised leaf explants cultured on basal MS medium. Excised leaf explants inoculated on Knop's + 2% S medium produced minimal number of buds.