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In Vitro Propagation of *Drynaria quercifolia* (L.) J. Sm., a Medicinal Fern.

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Abstract

Drynaria quercifolia is a highly medicinal fern used traditionally all over the world to treat a variety of ailments. But due to indiscriminate collection and destruction of habitats, it is facing the verdict of being endangered. So it is high time to adopt the various conservation measures including its in vitro propagation. In order to conserve the species in situ, in vitro culture of spores of the plant was tried.

The spores were found to germinate only on MS-Z4 medium (Full MS+1mg/L IAA+5mg/L Kinetin+20%CM+300mg/L CH). All other media used failed to produce prothallus. After inoculation, it took about 30 days to germinate. After development of the Prothallus and subsequent development sporophytes, the plantlets were transferred in different media but the best growth was observed in PNT-2 media (Full strength Parker and Thompson +5mg/L IAA+2mg/L Kinetin) where proliferation of vegetative fronds and rhizome were recorded.

Key words: *Drynaria quercifolia*, medicinal, *in vitro* propagation.

Introduction

Drynaria quercifolia (L.) J. Sm. is a highly medicinal fern which is in use among the masses to cure various ailments related to a variety of ailments. In Sanskrit it is called as *Ashvakatri*, its rhizome is bitter and astringent and used to treat bone fracture, cough, headache and typhoid fever. The fronds are administered for treatment of phthisis, fever, dyspepsia and cough in Malaysia. In India, Asolkar et al., (1992) reported its use in the treatment of body ache, rheumatism, skin diseases, and as a tonic, expectorant and anthemintic.

In Barak Valley of Sothern Assam, it is commonly called as *Uphatkarul* and is used to treat tuberculosis and throat infections (Sen et al., 2008). Though the plant is reported to be common in Barak Valley in general as well as in India, but due to heavy destruction of its habitat which includes big trees where it grows epiphytically, the future is not too far where this plant would also be included in the endangered list as it has already entered the same status in other parts of the world (Hegde and D'Souza, 2002). Keeping this in view, the above plant was selected for *in vitro* propagation in the present study.

Though the medicinal properties of *D. quercifolia* (L.) J.Sm. had been enlisted by various workers; no much work of the present kind was initiated. Hegde and D'Souza (2003) investigated the influence of media on the *in vitro* regeneration of the fern *Drynaria quercifolia*. In 2000, the same author together with L D'Souza produced *in vitro* sporophytes from the rhizome of *Drynaria quercifolia.* No other citation could be gathered on the topic.

Materials and Methods

Mature spores were collected from fertile fronds of *D. quercifolia* (L.) J.Sm. from Tilvum Reserve Forest of Karimganj District and air dried in oven at 30ºC for three days and consequently stored in sterilized glass bottles at 10ºC. Surface sterilization of spores were done with 35% (w/v) solution of Sodium hypochlorite (4% active chlorine) for 15

minutes and filtered through autoclaved filter paper (Mazumder et al, 2010). The spores were consequently washed several times with autoclaved distilled water to remove any traces of the chemical used and inoculated in medium in wet conditions.

Different media were used for the experiment. The standard Murashige & Skoog medium, Parker and Thompson medium, Knudson C medium were used along with their different strengths and combinations. The spores were transferred in aseptic conditions in Laminar Air Flow hood and incubated in Growth Chamber of the Tissue culture laboratory at 25ºC±1ºC under 16 hrs photo period at 2500-3000 Lux.

After every two weeks, the cultured materials were transferred into new medium for better growth of plants.

For acclimatization, a variety of hardening media were used comprising sterilized charcoal, brickbats, mosses along with dried leaves or water hyacinth, coconut fibers, and leaf molds in some cases. Small pots were used to transfer the plantlets in aseptic condition and kept in the tissue culture room for a few weeks followed by transferring in outer environment. Following combinations of potting mixtures were tried for acclimatization of the culture plants.

(a) Brick bats + Charcoal + Leaf mold — **C-1** (1: 1:1).

(b) Brick bats + Charcoal+ Dried water hyacinth— **C-2**(1:1:1)

(c) Brick bats + Coconut fiber + Sand— **C-3** (1:1:1).

(d) Brick bats + Charcoal+ Dried moss— **C-4** (1:1:1).

(e) Brick bats + Charcoal + Dried moss+ Leaf mold—— **C-5** (1:1:1:1).

The earthen pots containing the aforesaid potting mixture were autoclaved at 20 lbs/sq inch, pressure for 30 minutes. After sterilization the pots were allowed to cool down for 48 hours and then kept in room temperature in a well ventilated room having sufficient diffused sunlight.

Plants were taken out from culture flasks and washed thoroughly with water to remove culture medium sticking to the plants. After that the plants were dipped in 2 % Diethane 45 (Fungicide) for 30 seconds and transplanted in pots containing sterile mixture and covered with polythene bags. The pots were kept in tissue culture laboratory. The seedlings were regularly sprayed with MS solution up to 15 days of transfer and during the next 15 days these were sprayed with half strength MS solution.

After one month the seedlings were transplanted to community pots containing any one of the above five mixture combination. The plantlets were sprayed with Multiplex (1 ml/3 liters of water) solution fortnightly.

Results

The spores were found to germinate only on MS-Z4 medium (Full MS+1mg/L IAA+5mg/L Kinetin+20%CM+300mg/L CH). All other media used failed to produce prothallus. After inoculation, it took about 30 days to germinate. After prothallus growth, the plantlets were transferred in different media but the best growth was observed in PNT-2 media (Full Parker and Thompson +5mg/L IAA+2mg/L Kinetin) where proliferation of vegetative fronds and rhizome were recorded. In this study spores germinated well in the medium containing no sugar but Sheffield et al., 2001 cultured spores of *Pteridium aquilinum*, *Athyrium filix-femina*, *Dryopteris expansa* and *Anemia phyllitidis* in media containing sucrose. Percentage germination of all four species was significantly enhanced by the inclusion of sucrose.

Regenerated plants were transferred in the sterile vermiculate mixture for 1 month so that they can acquire better survival strength. After one month the plants were transferred to potting mixtures (as stated in Material & Methods) combinations. Maximum survival percentage was observed in the mixture containing Brick bats+ Charcoal+ Dried moss +Leaf mold (1:1:1:1), (Mazumder et al,2010).The survival percentage is given in Table No.1.

Table No.1: Survival Percentage of Sporophytes.

Potting mixture	Survival percentage (%) after 2months	Survival percentage (%) after 3 months
C-1	40	35
C-2	56	43.25
C-3	30	17.1
C-4	78.5	63.4
C-5	83.9	75.5

From the above table it is evident that the potting mixture combination C-5 is best for survival as well as hardening of *in vitro* cultured sporophytes (73.3%), followed by C-4 (65.4%). Mixture no. C-3 showed very poor results (17.1%) after 6 months of transplantation.

Discussions

The spores of *D.quercifolia* (L.) J. Sm. germinated in MS media supplemented with Auxin (IAA), Kinetin, coconut milk, and casein hydrolysate without sugar. Growth and proliferation needs sugar in the medium, but germination of spore is generally inhibited by the presence of sugar in the medium (Renner and Randi, 2004). It was also seen that supplementation with hormones and casein hydrosylate increased the germination capability of the spores. Same observations were also made by Mazumder et al., (2010) who worked with the *in vitro* propagation of *Bolbitis costata* (Wall ex Hook.) C. Chr.

Regarding callus formation and proliferation, it was seen that Parker and Thompson medium supplemented with Auxin (IAA) and Kinetin proved to be the best. Auxin alone leads to cell enlargement while Kinetin induces cell division in presence of auxin (Steward and Shantz, 1955). Cytokinin also helps in lipid metabolism and aids in growth (Manning and Van Staden, 1987). Similar results were also reported by Mazumder et al., (2010). Morini, (2000) and Fernández, et al, 1997 cultured spores of *Osmunda regalis* and have the same observation. Kuriyama, et al., (2004) reported about the production of sporophytic plants of *Cyathea lepifera*, in half strength MS medium with out sugar.However, addition of sugar enhance growth but failed to produce sporophytes. When diluted MS medium (1/2,1/20,1/40,1/80) without sugar was successful in initiating sporophytes from heart shaped Prothallus.

The plants were found to be ready for transplanting in hardening medium after about 12 months from the date of inoculation of spore. The plantlets were transferred in small pots containing vermiculite (a sterile inert medium for planting transferred plants) and kept inside the tissue culture room for acclimatization before exposing to the natural environment and sprayed with liquid PNT medium in a sequence of 2 times everyday. To ascertain the humidity, transparent polythene bags were used to cover the pots. Jagganathan (1985) also stressed upon the maintenance of humidity to avoid desiccation. After about two months, the plantlets were transferred to acclimatization media and it was seen that the best medium which served the purpose comprised of pre sterilized Brick bats + Charcoal + Dried moss+ Leaf mold. Leaf molds provided nutrition, while coconut fibers acted as the retention medium for water. Charcoal and brickbats were supplements for soil. Mazumder *et al* (2010) also obtained good results using similar medium, where fibers were replaced with moss, other components remaining the same. Saifulla et al., 2008 cultured *in vitro* regenerated plants of *Asplenium nidus* and acclimatized them in green house using different potting mixtures of garden sand, farmyard manure and charcoal and got very good result. The main points that should be kept under consideration while preparation of potting medium

other than nutritive value are water retention capability, sterilization and disinfection, avoiding water logging and good aeration.

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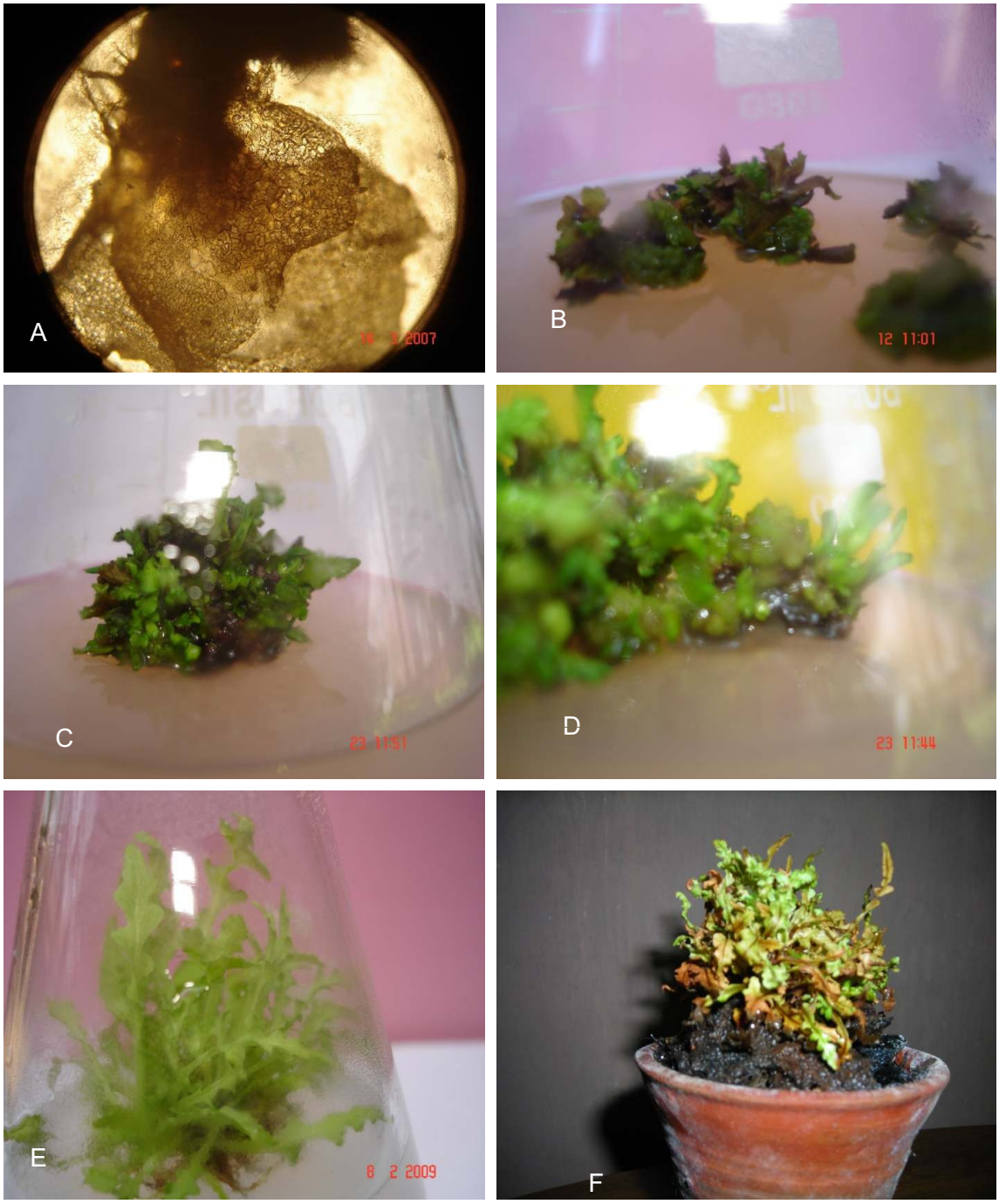

Plate 1: [A.] A developing Prothallus. [B, C,D.] Different stages of Sporophytes development from Prothallus. [E].Mature sporophytes after 6months of culture. [F].Acclimatization of plant in natural environment.

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