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Research Article

STUDIES ON EFFECT OF 2,4-D (2,4-DICHLOROPHENOXY ACETIC ACID) ON *IN VITRO* MORPHOGENESIS FOR CLONING AND CONSERVATION OF *MARSILEA QUADRIFOLIA* L. A FERN WITH ANTI HUMAN BREAST CANCER ACTIVITY

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ABSTRACT
Marsilea quadrifolia is a perennial creeping aquatic fern belonging to family Marsileacae. The genus *Marsilea* is widely distributed in temperate as well as tropical regions of the world. *M. quadrifolia* is one of the ten species reported from India. It has been assigned endangered status in Europe. The aquatic fern possesses pharmaceutically significant active principles. Keeping these facts in view, *in vitro* morphogenetic studies have been undertaken for establishing tissue culture protocols for micro propagation and conservation of *M. quadrifolia.* In the present study, 5-8 multiple shoots have been obtained from rhizomatous nodal explants cultured on Murashige and Skoog's (1962) medium supplemented with 2,4-D (2.26µM - 22.6 µM). Significant differences in morphogenetic response of aquatic and semi aquatic or land forms have been observed.

Keywords: *Marsilea Quadrifolia, Aquatic Fern, In Vitro, Micropropagation, 2,4-Dichlorophenoxy Acetic Acid (2,4-D)*

INTRODUCTION
Marsilea quadrifolia L. is one of the rapidly disappearing species having protected status in the international documents and lists concerning conservation (Brezeanu and Banciu, 2009). In developing countries including India the receding wetlands and water bodies (Chaturvedi, 2014) have caused alarming loss of *Marsilea* which was normally abundant earlier. The aquatic fern belongs to family Marsileaceae.

The genus *Marsilea* includes 65 species which is widely distributed in temperate as well as tropical regions. Most of the species occur in Australia and about ten species have been recorded from India. Morphologically, *Marsilea* has a slender creeping, dichotomously branched rhizome of indefinite growth, which may form a mat extending up to 25 meters in diameter. The plant is commonly called Araikeerai and Neeraral in Tamil and Malyalam respectively, while it is called Sunsuniya (Sushnisaag) in Hindi.

For centuries, people have been using plants for their therapeutic values (Singh *et al.*, 2011; Raageeva Bimal *et al*., 2011). Today 85,000 plants have been documented for therapeutic use globally as antimicrobial and cytotoxic agents from plant sources which may be easily accessible and might be cheaper with minimal side effect.

The fern may be found growing at the edges of ponds, canals and in wet or water logged areas. The leaves either grow erect as in land forms or float in submerged forms. It contains pharmaceutically active principles such as tannins, flavonoids, alkaloids, phenols, glycosides, terpenoids, saponins, sugars and steroids (Mathangi *et al.*, 2012; Shirolkar *et al.*, 2014) rendering the fern rich in medicinal properties. *M. quadrifolia* has been used as diuretic, febrifuge and antidote for snakebite (Duke and Ayensu, 1985). Very recently, the fern has been reported to exhibit anti proliferative activity against human breast cancer adenocarcinoma (MCF-7) cell lines (Uma and Pravin, 2013) and antimicrobial activity (Gini and Jothi, 2015).

According to Brezeanu and Banciu (2009) *M. quadrifolia* is one of the threatened species in Europe and has been assigned protected status in the red list and other international documents. Keeping these facts in

view, *in vitro* morphogenetic studies have been conducted for establishing tissue culture protocols for micro propagation and conservation of *M. quadrifolia.*

MATERIALS AND METHODS

The land and water forms of *M. quadrifolia* were collected from B.R.A. Bihar university campus Muzaffarpur and washed thoroughly under running tap water for 1-2 hours.

After proper washing healthy nodal parts, leaves and sporocarps were excised for further washing and surface sterilization.

The explants were treated with detergent solution for 30 min, followed by thorough washing of detergents. Later, the explants were further sterilized with 0.1% $HgCl_2$ for 10-15 min with continuous shaking.

Finally, explants were washed 5-7 times with double distilled sterile water. The properly sterilized explants were inoculated on MS, ½ MS and ¼ MS medium or medium supplemented with plant growth hormone. The cultures were transferred to culture room maintained at 25ºC ± 2ºC under cool, fluorescent with 16 hr photoperiod.

RESULTS AND DISCUSSION

The rhizomatous nodal explants of both land and water form of *M. quadrifolia* were cultured on MS plain medium with sucrose. Such explants did not show any visible morphogenetic response in any of the two types of explants (Figure 1 and Figure 2). The nodal explants cultured on different salt strength of MS medium as well as different concentrations of 2,4-D showed visibly different responses either in terms of proliferation or morphogenesis. The nodal explants cultured on ½ MS medium also did not show any morphogenetic change.

On the basis of our observations during experiments, MS (1/4 strength) was selected as the basal medium for further morphogenetic experiments because the cultured explants did not turn brown and also showed proliferation of multiple shoot initials in both the explants.

MS (1/4 strength) medium supplemented with 2,4-D (2.26µM) responded differently in two different forms of *M. quadrifolia*. The nodal parts of the land forms of *M. quadrifolia* showed 1-3 shoots with roots white in colour (Figure 3).

Out of three shoots, one was very tall in comparison to other two. At the same concentration, the cultured nodal explants of water form performed better and produced 2-5 shoots (Figure 4). In this case too, the roots were white. The morphogenesis of shoots is better in water form in terms of length and size of the shoots.

The nodal explants of land forms cultured on MS (1/4 strength) basal medium supplemented with 2,4-D (4.52 µM) proliferated 5-6 multiple shoots in two to three days of culture (Figure 5). Out of five shoots three shoots were taller than the rest of two shoots.

More interesting, the cultured nodal explants of water form produced either one, two or three shoots only. Further, the nodal explants cultured on MS (1/4 strength) supplemented with 2,4-D (11.31 µM), the explants from land form of *M. quadrifolia* showed proliferation of 2-3 multiple shoots in 3-4 days of culture (Figure 6), while no morphogenetic response was observed in cultured nodal explants of water form.

The higher concentration of the hormone 2,4-D decrease the shoot proliferation in the cultured nodal explants of *Marsilea*.

Allsopp (1952) also reported that *Marsilea* showed better growth performance in liquid medium. The differences in sensitivity of the cultured explants of land and water forms to hormone (2,4-D) seems to depend on the water balance of the tissue (Allsopp, 1955).

Elongation of *M. quadrifolia* is also promoted by submergence and may be attributed due to C_2H_4 formation (Chernys and Kende, 1996).

Thus, it seems that habit and habitat affect the sensitivity of cultured nodal explants of *M. quadrifolia* to the hormone 2,4-D modulating morphogenesis quite differently.

PLATE

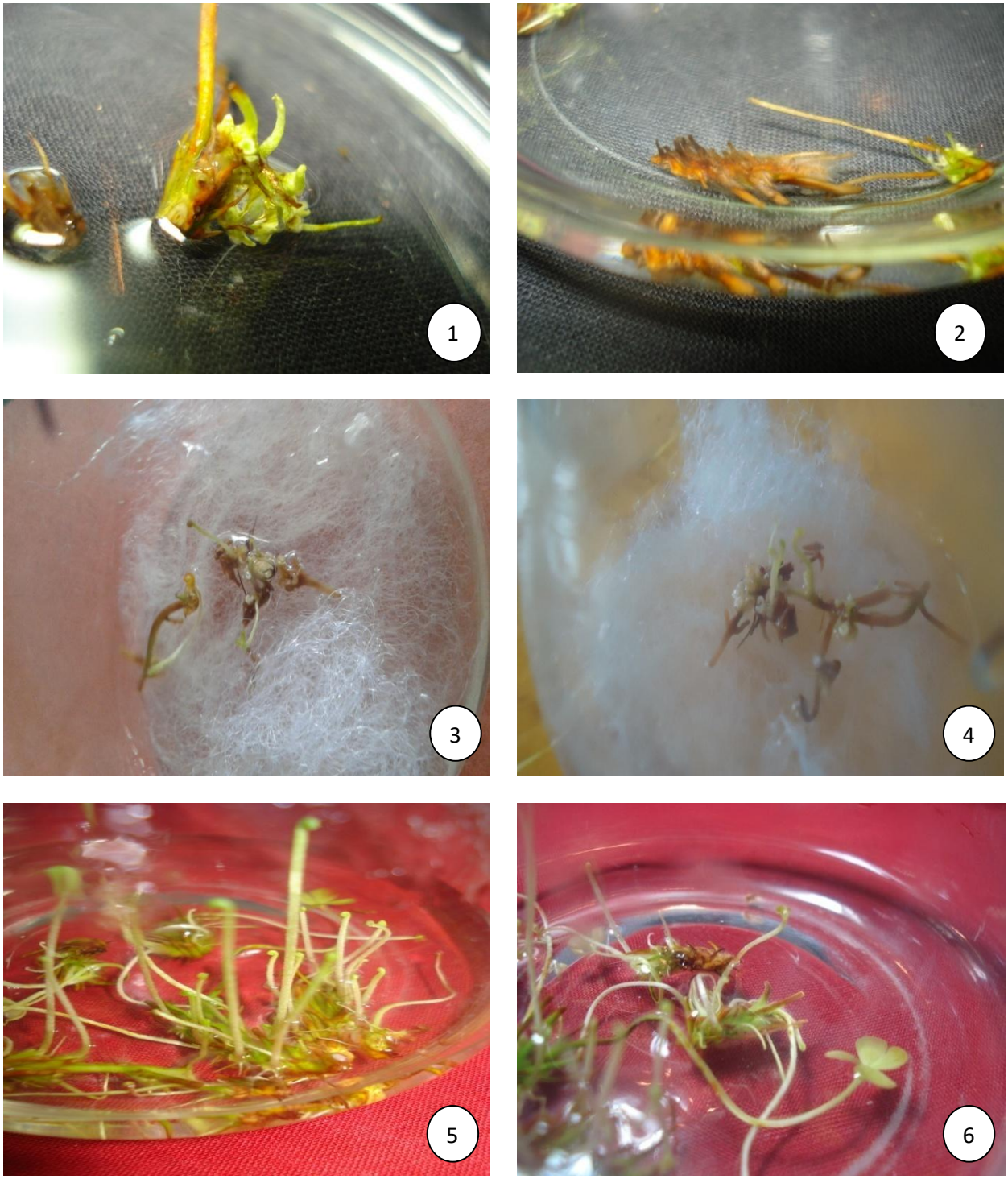

Figure 1: Nodal Explant of *M.quadrifolia* (Land Form) Cultured on MS Medium; Figure 2: Nodal Explant of *M.quadrifolia* (Water Form) Cultured on MS Medium; Figure 3: Multiple Shoots of *M.quadrifolia* (Land Form) on 1/4MS Plain+2,4-D (2.26µM); Figure 4: Multiple Shoots of *M.quadrifolia* (Water Form) on 1/4MS Plain +2,4-D (2.26µM); Figure 5: Well Differentiated Multiple Shoots of *M.quadrifolia* (Land Form) on 1/4MS +2,4-D(4.52 µM); Figure 6: 2-3 Well Differentiated Multiple Shoots of *M.quadrifolia* (Land Form) on ¼ MS+2,4-D (11.32 µM)

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