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STUDIES ON EFFECT OF BENZYL AMINO PURINE ON *In Vitro* MORPHOGENESIS IN *Marsilea quadrifolia* L.

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Key words : *Marsilea quadrifolia*, morphogenesis, BAP (Benzyl amino purine), tissue culture, water fern.

Hormonally regulated *in vitro* morphogenetic system has been established for clonal propagation of *Marsilea quadrifolia* L. in both aquatic and land forms. Shoot differentiation from rhizomatous explants have been observed on 1/4th strength Murashige and Skoog s(MS) medium (1962) supplemented with BAP (Benzyl Amino Purine). The shoot differentiation started in 2-3days of culture on L MS medium containing 0.5 mg/l BAP in land forms, while proliferation started in aquatic forms on L MS medium containing 0.1mg/l to 5mg/l BAP. Seasonal variation in morphogenetic response of cultured explants is significant.

INTRODUCTION

Marsilea quadrifolia L is a member of pteridophyte belonging to family *Marsileaceae*. In English it is commonly known as 'European Water Clover' while popularly known as Sunsuniya (Sushnisaag) in Hindi. Although, it is cosmopolitan in distribution, depends strongly on high moisture for sexual reproduction (Sekhawat and Manokari, 2015). *M. quadrifolia* is a favorite system for studying role of growth regulators, because it exists in nature both as land form as well as water form. Several studies have been made in the past to study the transition from land form to water form and *vice versa* (Allsopp, 1952,1955) but so far no intermediate transitional stages between land form and water form have been reported. The present paper describes the effect of BAP on *in vitro* morphogenesis in *M. quadrifolia*.

MATERIALS AND METHODS

The land and water forms of *Marsilea quadrifolia* L. were collected from B.R.A. Bihar University campus Muzaffarpur and washed thoroughly under running tap water for 1-2 hours. For tissue culture of nodal explants the protocol of Bimal et al. (2017) was followed. Properly sterilized explants were inoculated on MS, half strength MS and quarter strength MS medium either alone or supplemented with various concentrations of the hormone Benzyl Amino Purine (BAP). The cultures were maintained at 25°C ± 2°C under cool fluorescent light with 16 hr photoperiod.

RESULTS AND DISCUSSION

The effects of different concentrations of BAP on cultured explants of *M. quadrifolia*, have been recorded for both water form (Figs.1-3) and land form (Figs.4-6). In aquatic or water form almost all cultured explants were responsive and 9 shoots differentiated from rhizoidal explants cultured on ¼ MS containing 0.1mg/l BAP . The shoots were on the average 1cm in height. Six rhizoidal clumps from land form of *Marsilea* were inoculated in each flask on ¼ MS medium supplemented with 0.1mg/l BAP. The greening of explants (tissues) were observed which were supposed to be pre-requisite to the morphogenetic responses in culture. In land form 2-3 shoots developed on 4th day of culture but the growth and development was very slow in comparison to growth and development of shoots in the presence of 0.1mg/l BAP in aquatic forms. In the presence of 0.5mg/l BAP the cultured rhizoidal clumps showed greening of more tissues in the explants. 6-shoots differentiated from 8 explants cultured in each flask. The leaves opened in 3-4 days and also further elongation of shoots were observed. 5-7 shoots differentiated in 3 days and developed approximately 7 shoots per explants resulting in almost 30 shoots per flask. The basal parts became green and shoots originated from these greenish basal meristematic tissue centers. The elongation of shoots were maximum. In six days of culture, explants from land form developed 15 shoots and 22 shoots in the presence of 0.1mg/l and 0.5mg/l BAP respectively, while the explants from water (aquatic) from differentiated 9 shoots of 1cm height in average and 27 shoots with average 2cm height in the presence of 0.1mg/l BAP and 0.5mg/l BAP respectively. The responses of rhizome explants of land form were very slow and hardly one or two shoots were observed and also leaves were yellowish in colour in the presence of 1mg/l BAP.

In aquatic forms, the cultured rhizomatous explants in the presence of 2.5 mg/l BAP showed morphogenetic responses after a week of culture and 2-3 shoots were observed growing with small petiole and leaf turning pale yellow in colour. The morphogenetic response was poor in the rhizomatous explants in the presence of BAP (2.5mg/l) of land forms showing one thick shoot like structure, however, the basal parts showed green masses which did not develop further. The explants from aquatic forms showed 2-3 proliferated shoots with well developed leafy structures.

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In the presence of 5mg/l BAP the cultured nodal explants from aquatic forms showed typical development as if growing in natural habitat but the number of shoots were 3-4 per nodal explants. Leaves were green and fully open. The cultured rhizomatous explants of land forms showed the best response in terms of number of responsive explants, number of shoots and reproductive efficiency.

The *M. quadrifolia* explants have not been found to be morphogenetically responsive on full strength MS medium and turned black after one week of culture (Bimal et al. 2017). However, Shekhawat and Manokari(2015) reported multiple shoots formation on full strength MS medium supplemented with BAP. Half-strength MS medium was used by Brezeanu and Banciu (2009) and Rolli et.al. (2015) in their experiment with *M. quadrifolia* micro propagation. Shekhawat and Monakari (2015) reported delay in response and also lower number of shoots on ½ strength MS medium as also observed in the present experiment. In the present experimental system ½ strength MS was also not found to be the best nutrient medium for morphogenetic response. It seems likely that the *M. quadrifolia* ecotype used in culture required nutrients in much lower concentration and we observed flourishing growth of *M. quadrifolia* nodal or rhizomatous explants in ¼ strength of MS medium. Various cytokinins have been suggested to regulate physiological and developmental processes in lower and higher plants as well as in aerial or subterranean parts of plants such as cell division, chloroplast differentiation, auxiliary bud proliferation etc.

BAP (6-Benzyl Amino Purine) was reported to perform well in inducing microshoots in culture both in terms of % response as well as number of shoots in *M. quadrifolia* (Shekhawat and Manokari, 2015). Most of the studies on fern micro propagation depends on use of spore mediated parts as source for culture (Nakamura and Maeda,1994,; Fernandez *et.al*. 1999, Kuriyama and Maeda 1999; Cheema, 2005). In the present study shoots were hormonally induced from the nodal rhizomatous parts. Shoots were regenerated from explants in the presence of BAP alone, however, we have reported multiple shoots formation in the presence of 2,4-D earlier (Bimal et.al. 2017). Shekhawat and Manokari (2015) also used rhizomatous and nodal parts for culture and shoot regeneration.

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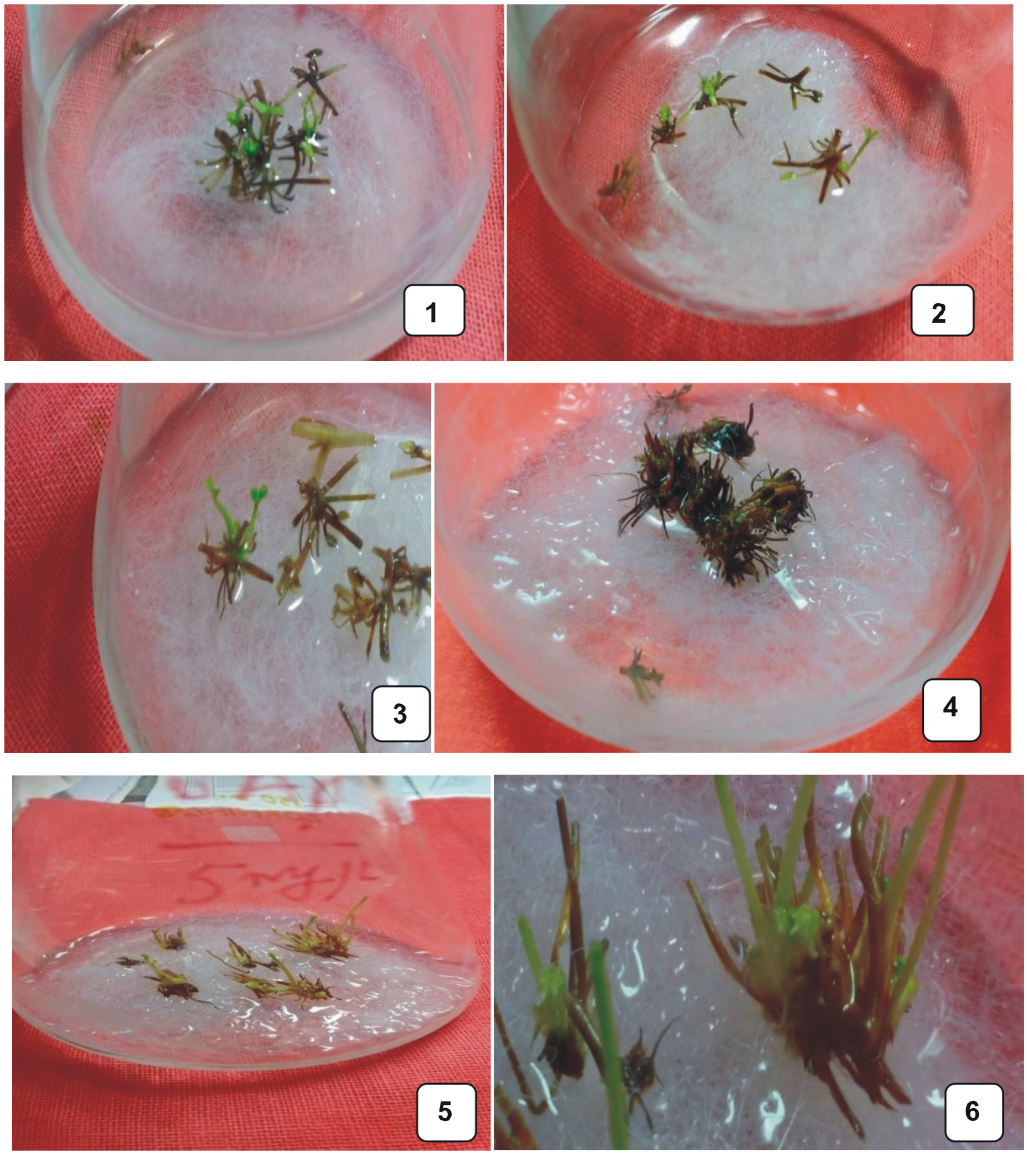

Legend : Photographs showing effects of BAP on growth and development in . Fig.1. Proliferation of shoots from nodal explants of aquatic forms cultured on ¼ MS+BAP (0.1mg/l). Fig.2. Shoots developing from nodal explants of aquatic forms cultured on ¼ MS+BAP (0.5mg/l). Fig.3. Development of tiny shoots from nodal explants of aquatic forms cultured on ¼ MS+BAP (5.0 mg/l). Figs.4-6. Rhizomatous clumps of land form of showing morphogenesis on ¼ MS +BAP 0.1mg/l, 0.5mg/l and 5.0mg/l respectively.