In Vitro Morphogenesis Of Colysis Latiloba (Ching.) Ching

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Abstract: - Spores of the fern *Colysis latiloba* reared in Knop's medium with various concentration of sucrose ranging from 0 to 6 % germinated after two weeks. Only 15% spores germinated in the medium without sucrose. Maximum spore germination 86% was observed in the medium with 1% sucrose. Pre-germination stages were almost similar in all the cases. This species exhibited bipolar germination. The gametophyte was thalloid, dorsoventrally flattened and spatulate. Sex organs were not observed up to 23 weeks, which indicates development of apogamous sporophyte.

Index Term: - *Colysis latiloba,* Ptheridophytes Morphogenesis, Knop's Medium, Apogamous Sporogophyte

1 INTRODUCTION

Pteridophytes are interesting group of plants possessing long evolutionary history. The giant lycopods form dominant vegetation during Devonian times. In evolutionary history, pteriodophytes play important role holding intermediate position between nonvascular plants, the 'bryophytes' andthe seed bearing plants the 'spermatophytes' (Rashid, 1999). Globally 11, 300 species of pteridophytes are reported (Wilson, 1992). An enumeration of pteridophytes compiled by Iwatsuki (1988) reported 380 species from Nepal. According to Thapa (2002), 534 species of Pteridophytes under 102 genera belongs to 35 families are reported from Nepal. Numerous works have been carried out in higher groups of plants (angiosperms and gymnosperms), while research in the lower groups of plants in general has not been carried out seriously and systematically (Chaudhary, 1998). Ferns are considered to be one of the most favorable materials for growing on the nutrients media under controlled condition of aseptic culture. They quick respond to changes in that condition. Moreover, they are easy to handle in large numbers and may readily be put into sterile culture starting from spores. Some of studies in which fern gametophytes have been used include investigation of growth correlation, regeneration and hormonal effects (Albaum, 1938a, 1938b); the effect of environmental gradients (Naf, 1953); the induction of sex organ formation (Naf, 1956, 1958); the effect of the inhibition of protein systhesis on growth (Hotta and Osawa, 1958); induction of apogamous sporophyte development (Whittier and Steeves, 1960, 1962; Mehra and Sulklyan, 1969; Kato, 1970); effect of sucrose on heteroblastic leaf development (Sussex and Clutter, 1960; Caponetti, 1970) and many more. Pteridophytes have attracted several researchers for morphogenetic studies. Morphogenesis deals with the development of organisms and their parts from a single cell.

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Their structural simplicity as compared to flowering plants, relative case of two distant independent altering sporophytic and gametophytic generations is responsible for their appeal to the investigations. Both the sporophytic and gametophytic generation develops from single celled zygote and spore respectively. By suitably manipulating the cultural condition *in vitro*, it has been possible to induce apogamous sporophytes and aposporous gametophyte without the intervention of fertilization and sporogenesis. Thus, pteridophytes offer a vast scope of morphogenetic studies in that the experimental work can go well beyond the mere callus formation and differentiation and also cover vis-à-vis nutritive environment Such studies have been found useful for several works on experimental pteridology. Nutritional studies on fern have highlighted their response to cultural condition (Bir, S. S. *et al*., 1982). Fern system has proved to be an excellent material for the study of the alternation of generation. However, the alternation of generation is eliminated by vegetative reproduction. The regular alternation of generation can also be altered by various factors as nutrition, light, and physical factors, which play a regulating role in the various stages of the orderly development of gametophytic and sporophytic generation during the fern life history. Moreover, it is well known that cyclic alternation of generation can also be circumvented through the occurrence of apogamy and apospory. *Colysis latiloba* belongs to the family Polypodiaceae of Leptosporangiate, is an ornamental species grown for indoor as well as outdoor decoration (fig. 1, 2). Frond is deep green, 25-60 cm long and 8-20cm broad. Lamina deeply cut to the main rachis to form a very beautiful lobe with rather linear, bright brown sori on either side of the main vein. It is terrestrial, usually occurring is shady moist forest slope and on rock crevices in 1250 to 2286m altitude in East and Central Nepal.

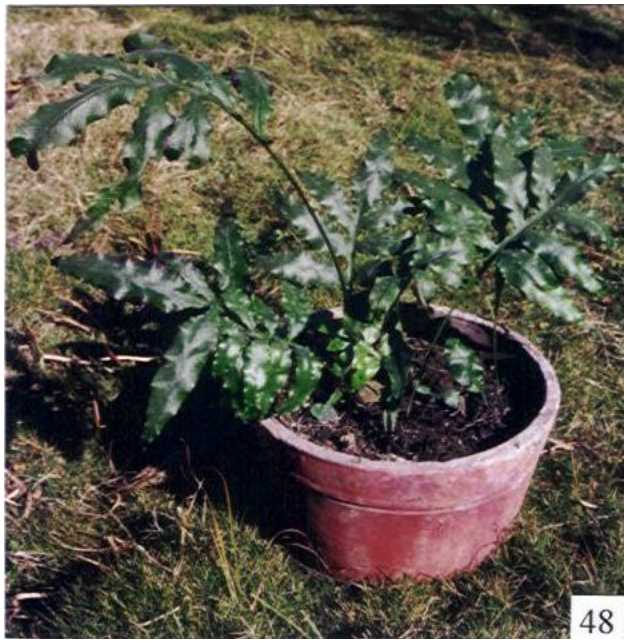

Fig. 1: *Colysis latiloba* growing in pot

Fig. 2: Frond showing brown linear sori, ventral view

Potting will be excellent with ordinary garden soil, compost and coarse sand, and should be kept in the shady place with moist condition. In vitro culture has been extensively carried out in various laboratories of the world for obtaining desired plants in short period. The threatened and endangered species, medicinal and aromatic plants (Pant et al., 1996), crop, fodder (Rout et al., 1994), and horticultural and ornamental plants can be propagated through In vitro technique. The ornamental fern Colysis latiloba was undertaken for the present study. The fern was multiplied in vitro through spores. The effect of various sucrose concentrations on the spore germination, sporeling development and gametophyte morphogenesis was studied.

2 MATERIALS AND METHODS

The materials used for the present study was the spores of Colysis latiloba (Ching.) Ching, collected from the garden of Central Department of Botany, Kirtipur, Nepal. Fresh spores were taken on season and dry spores were taken on offseason period.

I. Surface Sterilization

The mature fronds of Colysis latiloba bearing spores on their ventral surface were taken and washed serially with diluted detergent or teepol, running tap water for about half an hour, and with distilled water for 3-4 times .The pinna was cut into small pieces of 3-5mm and surface sterilized with freshly prepared 1% sodium hypochlorite for 12 minutes and finally rinsed with sterile distilled water for 5-6 times inside the laminar airflow chamber.

II. Spore Culture

Spores were taken in the sterilized petridish with the help of surgical blade pressing one side of the frond with the forceps. Then spores were inoculated on slanting Knop's medium supplemented with various concentration of sucrose. Concentration of sucrose varied from 0%, 1%, 2%, 3%, 4%, 5% and 6%. The medium was solidified by adding 0.7% agar. The pH of the medium was adjusted to 5.8 by adding either HCl or NaOH. All the cultures were maintained at 25o C (+2) illuminated by fluorescent lamp for 16 hours. The nutrient media used for the experiments was Knop's nutrient solution.

Chemical Composition of Knop's Basal Medium (KBM)

S.N	Chemicals	Chemical formula	Amount (mg/l)
1.	Calcium nitrate four Hydrate	Ca NO3. 4H2O	500
2.	Magnesium sulphate seven hydrate	Mg SO4. 7H2O	125
3.	Potassium nitrate	KNO3	125
4.	Potassium dihydrogen phosphate	KH2 PO4	125

3 OBSERVATION

Swelling, expansion and greening of a spore and rupture of the exine were the basic pre-germination changes. The spore responded towards the medium after one week of inoculation by swelling and intensification of dark brown colour. The spores became greener after two weeks. The spore germination was observed within four weeks of culture in all concentration of sucrose. Early stages of spores' germination were found same in all cases. However, mature gametophyte varied grown on media supplemented with various concentration of sucrose. In medium without sucrose, only 15% spores germinated. Eight-celled prothallus with five rhizoids was observed after seven-week as shown in fig. 3. Mature spatulate gametophyte was developed after 17 weeks. In the medium with 1% concentration of sucrose 86% spores germinated which was highest among the sucrose media tested. The prothallus containing eight rhizoids at different stages of development was observed after five week (fig. 4). Thalloid, dorsoventrally flattened, spatulate gametophytes were

observed after 17 week (fig. 5). The germination percentage of spores in medium with 2% sucrose was 75%. 10 cells long having four rhizoids prothallus was observed after five weeks (fig. 6). Spatulate gametophyte was observed after seven weeks. The cell of gametophytes contained dense chloroplasts and starch grain (fig. 7). In the medium with 3% sucrose, 67% spore germinated. A prothallus having eight cells and one rhizoid was observed after five week (fig. 8). The 17-week-old gametophyte contained dense rhizoids throughout the gametophyte body. A total of 63% spores were germinated in medium containing 4% sucrose. After four week, three-celled long protonemal filament containing single rhizoid was observed (fig. 9). Slightly rounded gametophyte with seven rhizoids at different stages of development was observed after seven weeks (fig. 10). Such type of gametophyte was observed only in KBM with 4% sucrose. Spatulate gametophyte containing large number of rhizoid throughout the gametophyte was observed after 17 weeks (fig. 11). The number of rhizoid was highest in KBM with 4% sucrose among the media tested. The germination of spore was 54% in medium containing 5% sucrose. Seven-celled prothallus with three rhizoids was observed after five weeks (fig. 12). Spatulate gametophyte observed after 11 weeks of culture is shown in fig. 13. In the medium with 6% concentration of sucrose 40% of the spores were germinated. Eight-celled prothallus with two long rhizoids was observed after five weeks (fig. 14). Spatulate gametophyte was observed after 11 weeks. In all cases, dorsoventrally flattened gametophyte with meristimatic cells at apical end were observed. The sex organs i.e. antheridia and archigonia were also not observed upto 23 weeks.

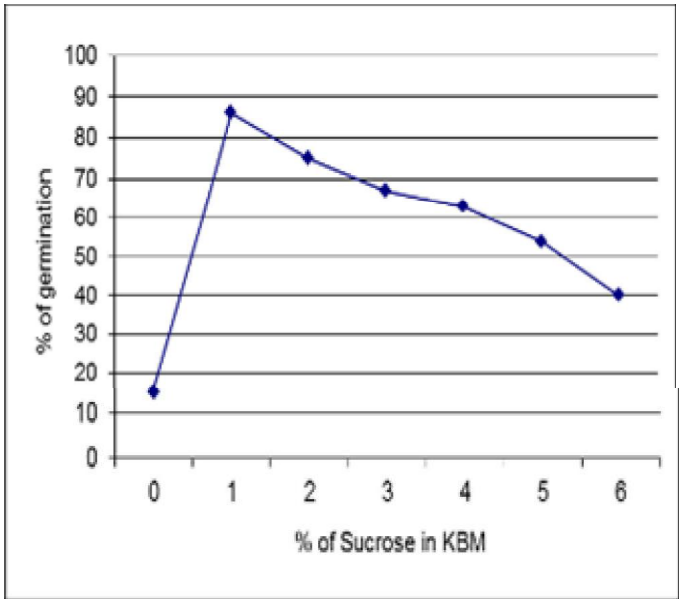

Fig. A: Effect of different concentration of sucrose in spore germination

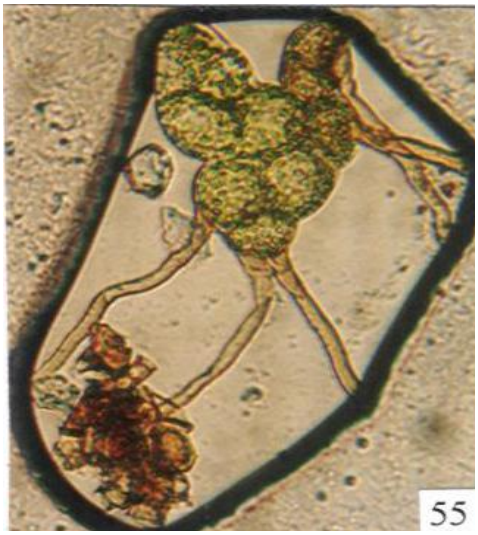

Fig. 3: Eight celled prothalus on KBM without sucrose after 7 weeks (x100)

Fig. 4: Prothallus on KBM with 1% sucrose after 5 weeks (x100)

Fig. 5: Spatulate gametophytes on KBM with 1% sucrose after 17 weeks (x10)

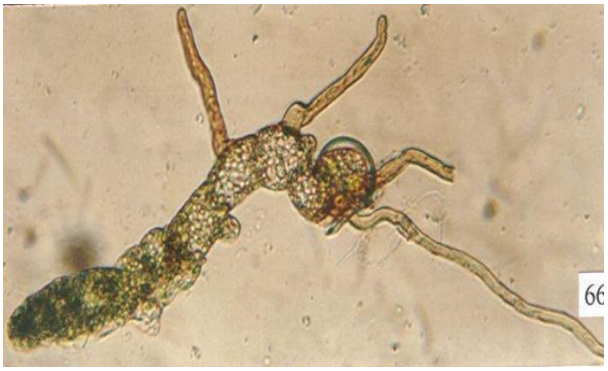

Fig. 6: Gametophytes on KBM with 2% sucrose after 7 weeks (x100)

Fig. 7: Prothallus on KBM with 2% sucrose after 5 weeks (x100)

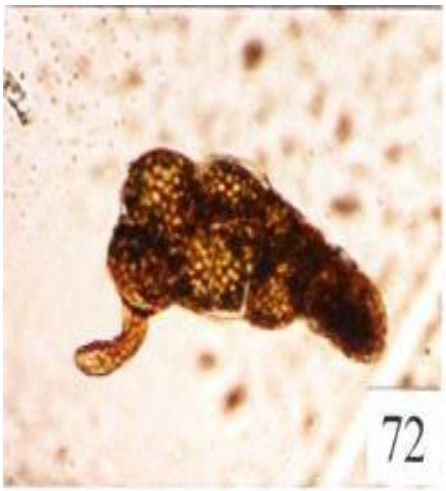

Fig. 8: Prothallus on KBM with 3% sucrose after 5 weeks (x100)

Fig. 9: Germinating spores with protonemal filament on KBM with 4% sucrose after 5 weeks (x100)

Fig. 10: Slightly rounded gametophytes on KBM with 4% sucrose after 7 weeks (x100)

Fig. 11: Gametophyte on KBM with 4% sucrose after 17 weeks (x10)

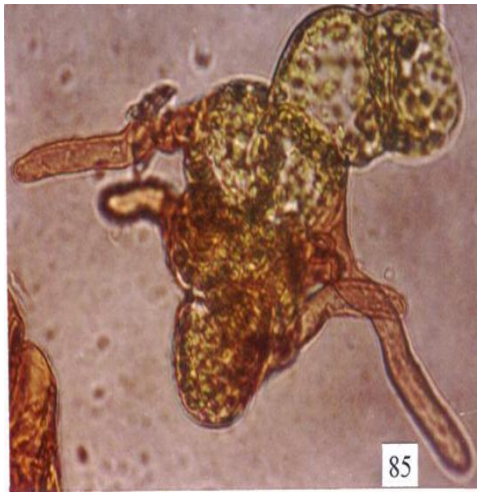

Fig. 12: Prothallus on KBM with 5% sucrose after 5 weeks (x400)

Fig. 13: Gametophytes on KBM with 5% sucrose after 11 weeks (x40)

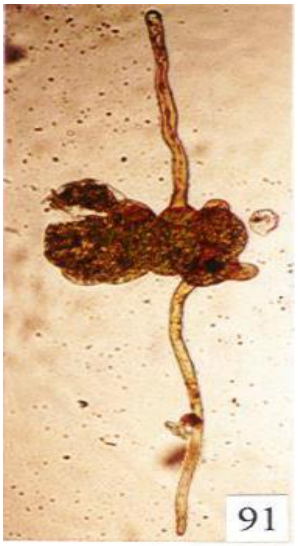

Fig. 14: Prothallus on KBM with 6% sucrose after 5 weeks (x400)

4 DISCUSSION

The fern spores require an adequate amount of moisture, a suitable temperature and period of dormancy for their germination like in many other plants both of higher and lower groups. In Colysis latiloba, the period of dormancy was found to be dependent mostly on the temperature and the composition of the cultural media. At higher temperature (30 + 2oC) the germination was much quicker (2 weeks) in contrast to 5 weeks at lower temperature (15+2oC). Conway (1949) has found that high temperature in the range of 30-35oC is best for spore germination. Hurel-Py (1944) reported the spores of Nephrolepsis cordifolia require adequate moisture for germination. Liquid media are more favorable than solid media. An adequate supply of moisture, a suitable temperature and pH range around 5.8 and availability of a sufficient light of suitable quality should be needed for the germination of the fern spore (Miller, 1968). In case of Colysis latiloba concentration of sucrose in the medium was effective factor on the germination of spores and gametophyte morphogenesis. At 1% sucrose medium highest 86% spores germination was observed. Further increase in sucrose concentration resulted in decrease of spore germination. This study confirms that low sucrose concentration has greater promotory effect than higher sucrose concentration. The study by Courbet (1957) also reported the lower concentration (0.5 % to 2.0%) of sucrose was promotory to spore germination of Athyrium filix femina. Ranjan (1979) also found KBM with 1% sucrose as the best medium for the spore germination of Cryptomium falcatum and Dryopteris marginata. He further concluded that higher percentage of sucrose is inhibitory to spore germination in both species. Devkota (1980) also obtained similar result; she reported that high concentration of sucrose inhibited spore germination. Joshi (1977) working on some species of Microssorium reported that high concentration of sucrose inhibited spore germination. But low concentrations of sucrose (0.25-1.5) were most favorable for spore germination. This suggested that spores of Colysis latiloba have ability to utilize wide range of sucrose concentration. The fall in spore germination percentage at higher concentration of sucrose may be due to the increase in osmotic pressure of the medium than DPD of the spore, thus lacking adequate moisture for spore germination. Gametophyte of the Colysis latiloba has a growth form quite different from that of the well-known heart- or butterflyshaped gametophytes such as are found in Pteridium, Cyathea or Onoclea. The principal difference is that the meristem never becomes confined to an apical notch but remains extended across the growing tip of the thallus. Derivatives of the meristem enlarge primarily in longitudinal direction and produce a spatulate gametophyte. The longitudinal division of the tip of the protonema resulted in a spatulate gametophyte (Nayar and Chandra 1963). In categorizing the types of gametophytes development in homosporus fern, Nayar and Kaur (1969) termed this pattern of development the “Kaulinia”. Similar result was also obtained by Farrar (1974) on different species of Vittariaceae. Presence of sugar, carbon source for vegetative growth under sterile condition in the medium has been reported as one of the causal agents in the induction of apogamy (Kato 1970, Mehra and Sulkalyan 1969, Whittier 1964a, 1964b). This study also supports for

apogamy as all the gametophytes of Colysis latiloba grown on 0 to 6% sucrose medium did not show the signs of developing sex organs over the period of 23 weeks. Similarly, Whittier and Steeves (1960, 1962) induced apogamy in Pteridium aquilinum by using 4.0% sucrose. They obtained greater response of apogamy at 5.0% sucrose. On the other hand, Devkota and Joshi (1980) reported development of apogamous sporophytes from gametophytes of Pteris quadriaurita growing on medium with 4.5 to 5.5 % sucrose. In Ampelopteris also 4.0% sucrose or 5.0% sucrose with 1 to 2mg/l 2, 4-D was found optional for apogamous soprophyte initiation (Sulkalyan and Mehra, 1971).

5 CONCLUSION

The results of the study suggested that the Knop's Basal Medium containing 1% sucrose was the best medium for the spore germination. High concentration as well as without sucrose medium was found inhibitory for spore germination. *Colysis latiloba* exhibited bipolar germination and the gametophyte was spalulate. The gametophytic development was Kaulinia type. Sex organs were not observed up to 23 week which indicates the development of apogamous sporophyte.

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