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ORIGINAL ARTICLE

Pteris vittata Propagation through Different Exposure of Chromium Concentration: an Experiment to Comprehend Phytoremediation Properties

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ABSTRACT
In a recent scenario of heavy metal contamination in soil, chromium (Cr) stays as one of prime contaminant in the environment because of its versatile anthropomorphic activities. Emphasizing phytoremediation as a major approach in eliminating heavy metal contamination, a tissue culture approach was designed in this study to understand the accumulation of chromium by Pteris vittata through supplementing different concentration of chromium in tissue culture media. We have studied the different stages of Pteris vittata in comparison with chromium treated spores. The result showed that Pteris vittata could able to sustain in concentration up to 20 ppm with some changes in its anatomical structure and the formation of spatulate stage and coordinate gametophyte stage was less as compared to the control environment. But in higher concentrations (≥ 20ppm) of chromium treatment, the growth of Pteris vittata was found to inhibit spore germination. Thus this study hypothesized that Pteris vittata has the ability to remediate the pollute amount of chromium from the environment and it can be used as a phytoremedial agent particularly concerning heavy metals contamination.
Key words- *Pteris vittata; chromium; tissue culture; Phytoremediation*

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INTRODUCTION

The major hazardous metals of concern for India in terms of their environmental load and health effects are lead, mercury, chromium, cadmium, copper and aluminium. With rapid industrialization and consumerist lifestyle, anthropogenic sources of environmental pollution have increased. Chromium compounds are highly toxic to plants and are detrimental to their growth and development. The pathway of Cr (VI) transport is an active mechanism involving carriers of essential anions such as sulfate [1]. Fe, S and P are known also to compete with Cr for carrier binding [2]. Cr (VI) has a high redox potential (1.33–1.38 EV) which is a strong oxidant with accounting for a rapid and high generation of ROS and its resultant toxicity [3]. Cr (III) is less toxic and for the most part bound to organic matter in soil and aquatic environments [4]. Naturally Cr is occurring at different concentration in soil (10-50mg. Kg^{-1}), seawater (0.1-117ugL^{-1}), and atmosphere ($5.0X10^{-6}$-$1.2X10^{-3}$ugm^{-3}) depending on parental material [5]. In India, about 2,000-32,000 tons of elemental Cr annually escapes into the environment from tanning industries. The leather industry is the major cause of the high influx of Cr to the biosphere, accounting for 40% of the total industrial use [6]. Hexavalent chromium compounds are used in industry for metal plating, cooling tower water treatment, hide tanning and, until recently, wood preservation. These anthropogenic activities have led to the widespread contamination that Cr shows in the environment and have increased its bio-availability and bio-mobility [7].

Phytoremediation is a promising method to remediate contaminated from soils. It consists of mitigating contaminant in polluted soils, water, or air, through plants able to degrade, or eliminate metals, and various other contaminants from the media that contain them. Ferns were recorded on serpentine soils high in nickel and chromium. It was noted that *Salvinia natans* absorb copper [8], *Helianthus annuus* L absorb chromium (VI) on phosphorus fractions [9] as well as *Salvinia minima* for chromium [10]. *Pteris vittata* L. has been reported to be a hyper accumulator of Arsenic [11]. The Chinese Brake Fern (*Pteris vittata*), the Spider Brake Fern (*Pteris multifida*), the Cretan Brake Fern (*Pteris cretica mayii*) and

several other *Pteris* ferns have been shown to uptake so much inorganic arsenic in soils that they can actually reduce the soil arsenic concentration of a moderately contaminated site to acceptable levels in just a few growing seasons [12]. An, Z. Z. described that the high Zn tolerance, relatively high ability to accumulate Zn, and great capacity to accumulate As under conditions of suppression by high Zn suggest that *P. vittata* L. could be useful for the remediation of sites co-contaminated with Zn and As [13]. A field study was conducted to determine the efficiency of Chinese brake fern, an arsenic hyperaccumulator, on removal of arsenic from the soil at an arsenic-contaminated site [14]. Natarajan demonstrated that *P. vittata* is effective in remediating As-contaminated groundwater to meet recommended standards [15].
Now days the increase amount of contaminant Cr in the environment is a very serious source of concern for plant and animal scientists due to the use of Cr in various anthropomorphic activities. In the context of the above it is urgently required to give attention on such valuable gift of nature and utilize it in pollution abatement and ultimately the social welfare by reducing the levels of pollutants from soil, water and air.

MATERIALS AND METHODS

Explant collections

Fertile fronds of *Pteris vittata* were procured from fern house, NBRI, Lucknow, India. The fertile pinnate was kept in brown paper packets and were stored in a desiccator. After spores were released, they were sterilized with 2 % sodium hypochlorite solution for minutes and rinsed with sterile distilled water.

Plant propagation

Surface sterilized spores were sown onto the Parker's and Thompson's basal nutrients medium solidified with 1% agar in glass petri dishes. The inoculation operation was performed under the laminar air flow chamber to check the contamination by microorganisms. These cultures were kept in culture room. The surface sterilized spores were inoculated into Parker and Thompson's media of full strength (P&T), half strength (P&T/2) and quarter strength (P&T/4). The different concentrations of growth regulators and vitamins viz. 2, 4-Dichlorophenoxy Acetic Acid (2, 4 - D), Indole-3-Acetic Acid (IAA), Benzylamine purine (BAP), α - Naphthalene Acetic Acid (NAA), Kinetin, Isopentenyl adenine (2ip), 2, 4, 5-tricholorophenoxy acetic acid (2, 4, 5 - T), thiamine HCl, pyridoxine HCl, m-inositol and trace elements were also added to the media. Various percentages of sucrose and agar were added and the pH was adjusted approx. 5.8 before sterilization in an autoclave.
The temperature of the room was maintained at 22 ± 2 ºC temperature and 2200 - 2300 Lux intensity. Light intensity was provided for 16 hrs light photoperiod followed by 8 hours dark photoperiod. The observation was made every alternative week. Periodically the spore germination and subsequent gametophyte growth were observed under the Nikon Trinocular microscope and the photographs were taken using a Nikon camera UF-II.

Statistical Analysis

To test the significance of experimental results, one way and two way Analysis Of Variance (ANOVA) were carried out to compare the mean values between different groups and results were expressed as mean ± standard deviation (SD).

RESULTS

Tissue culture of *P. vittata*

Besides the sexual mode of propagation involving the union of an antherozoid and an egg within the gametophyte and the growth of a sporophyte from a spore, ferns can also be propagated by means of a vegetative method known as tissue culture. Tissue culture is very useful technique to study the cell propagation with aseptic and artificial environment for contaminant. The spores of *Pteris vittata* germinated in Parker and Thompson medium supplemented with and without sucrose and hormone supplementation. The germination percentage of spore was maximal in Parker and Thompson medium with combinations of 2, 4-D at 2 mg/l, 2, 4, 5-T at 1 mg/l, and kinetin 2 mg/l. However the growth of sporophytes always requires sucrose in the medium. 30- 40 % Sodium hypochlorite with 4% active chlorine is suitable for sterilization [16]. Regarding medium, Parker and Thompson medium was found to be the best for germination of spores. In vitro culture of ferns will create a new path in the conservation work of ferns. Experimental results showed that the combination of 2, 4-D at 2 mg/l, 2, 4, 5-T at 1mg/l, and Kinetin 2 mg/l in P&T media with 1% sucrose was the most favourable dose for spore germination of *Pteris vittata*. The spores of *Pteris vittata* started germination about 7 days from the date of sowing. The rhizoids start emerging first followed by the green germ tube. The 2D stage was obtained

after 21 days from the day of sowing and the spatulate stage was observed after about 28 days. The cordate gametophyte stage appeared after approximately 35 days (Table 1).

Spore germination of *P. vittata*

To see the effect of chromium on spore germination, *Pteris vittata* were exposed to 10ppm, 20ppm, 30ppm, 60ppm and 100ppm of chromium concentration separately in P and T media (Table 1). After preparing petri dish, the spores collected were observed on the plate and then kept in the culture room. This observation showed that the spores of control as well as 10 ppm started germination in 7 days but percentage germination become reduced in plates supplemented with chromium concentration greater than 10 ppm to 30 ppm (figure 4A). While in plate having a concentration of 60 ppm, the germination started almost after one month. Although the petri dish having 100 ppm of chromium concentration also showed germination after one month but some cells are abnormally long ($P< 0.001$; Figure 1).

Rhizoid formation of *P. vittata*

It was noticed the average number of rhizoid increases in due courses of time with respect to increased concentration of chromium. The rhizoid number of controls was less as compared to chromium treated *P. vittata*. Rhizoid formation in control was observed from early seven days and this formation of rhizoid pattern was also found in 10ppm and 20ppm chromium treated spores (Figure 4A). However, 30ppm chromium treated spores, rhizoid formations was observed after fourteen days but in case of 60 and 100 ppm treated spores, the same was observed in quite late after twenty eight days. In comparison to control, it was observed that rhizoid formation increased progressively with increasing concentration of chromium in petri dishes (Figure 2).

Protonemal stage of *P. vittata*

The protonemal stage was taken in both one and dimensional views in the microscope. The two dimensional view in the microscope is more statistically significant than the one dimensional microscopic view. In twenty one days of observation two dimensional microscopic views were seen in control, 10 ppm and 20 ppm petri dishes. But in a higher percentage of treated chromium in 30 ppm, 60 ppm and 100 ppm petri dishes were started on or after twenty eight, thirty two and forty two days respectively (Figure 3). The observation between twenty one days to forty days the 20 ppm chromium treated petri dish had more number of the two dimensional protonemal cell growths as compared to rest of the experimented petri dishes (Figure 4B).

Spatulate and cordate gametophyte stage of *P. Vittata*

With repeated divisions in oblique or longitudinal, the apical cell gives rise to spatulate stage and further division make it cordate gametophyte stage (Figure 4C and 4D). These two stages were most important for the growth of *Pteris vittata*, in these stages actual difference came in control and chromium treated experimental petri dishes. It was observed from our result that the spatulate stage occurred on or after twenty eight days in control. The 10 ppm and 20 ppm environment were favourable as compared to other treated chromium concentration but some changes in anatomical structure of the *Pteris vittata* were observed. There was an inadequate growth of *Pteris vittata* was observed as indicated from cordate gametophyte stage in case of media supplemented with chromium concentration more than 20 ppm. Similarly the cordate gametophyte stage was observed from the thirty five days in control as well as 10 ppm experimented petri dish. There were some growth of cordate gametophyte stage in 20 ppm but at 60 ppm and 100 ppm cordate gametophyte stage was totally absent. The growths of the spatulate stage reduce sequentially with increasing the concentration of chromium and there was no formation of the spatulate stage at all in 60 ppm and 100 ppm (Table 2).

DISCUSSION

One recent study was proved that most of the trace elements were found in pteridophytes with the help of instrumental neutron activation analysis [17]. Chromium usually is a coexisting contaminant with arsenic in most contaminated soils. In this study, different concentration of chromium was exposed to *Pteris vittata* in the direction of analysis the germination period of the spore through tissue culture. In higher concentration of chromium the *Pteris vittata* germination of spore occurred at a later stage, it was after twenty eight days of the spore sown. In control spore germinated fully after forty two days but it was decreasing gradually as the concentration of chromium goes up. Chromium concentration of 10ppm was slightly different to the control, it was germinated after seven days but the percentage of germination of spores was less than control.

The rhizoid formation was abnormal in chromium treated spore except the control environment. The dwarfing and branching of rhizoids were mainly due to the broth-environment contamination. The

number of rhizoids increased with respect to increased chromium treatment to the petri dishes. At 10 ppm, 20 ppm and 30 ppm of chromium treated petri dishes the residues were more branched, septa were present and also showed bulbous base. Some cells of rhizoids were abnormally long in higher concentration (60 ppm and 100 ppm) of chromium treatment, and also the formation of rhizoids took place on or after thirty five days.

In the gametophytic generation of *Pteris vittata*, the germ filament represents a transient phase in anticipation of a major morphogenetic event, as, sooner or later, its terminal cell characteristically divides by an oblique or longitudinal wall to initiate planar morphology. Induction of planar growth in the protonema represented the beginning of a morphogenetic process that prepares it for the production of gametes. The first division of the terminal cell of the germ filament was oblique or longitudinal. This was followed by a partition at right angles to the first, generating a group of three cells. One of these, usually a wedge-shaped cell which occupied the central region at the tip of the filament, now functions as the meristematic apical cell. The formation of this cell marked the initiation of a process that produces a flat prothallus. By repeated oblique or longitudinal divisions with a left-right alternation of cell plate orientation, the apical cell gave rise to a spatulate mass of cells. At first, the apical cell appeared as a small indention at the tip of the spatulate plate, but later, as the two sides of the plate extended horizontally to assume a heart shaped form, the meristematic cell was safely lodged in the notch between the lobes and was referred as cordate. During further expansion of the lobes, the apical cell divided transversely followed by the division of the anterior cell by two or three walls parallel to each other. Thus a row of three or four narrow cells constituting a pluricellular meristem was born. From this point onward, division of cells of the meristematic plate accounted for the growth of the gametophyte. In the leaf-like prothallus, a tissue analogous to a midrib was formed by the division of cells behind the meristem. At this stage the prothallus was considered to have attained sexual maturity. In certain ferns there is very little change in the division sequences of the germinated spore up to the stage of formation of the heart-shaped gametophyte that its changing geometry readily yields to computer simulation [18].

Table-1 The table is showing the germination of spores from the first day of the sowing stage to the cordate stage of *Pteris vittata* in control environment.

S. No.	Days of observation	Mean % of spore germination	Mean no. of rhizoids	Mean no. of cells in 2D stage	Spatulate stage	Cordate stage
1	7	68.33	1.66	0	0	0
2	14	80.33	2.33	0	0	0
3	21	89.66	2.66	20.66	0	0
4	28	94.66	3.33	33.33	++	0
5	35	100	6.33	59.33	++	++
6	42	100	7.66	67.33	++	++

Table-2 The table is showing the germination of spore from the first day of the sowing stage to the cordate gametophyte stage of *Pteris vittata*.

Stages	Observation	Control	10ppm	20ppm	30ppm	60ppm	100ppm
Spatulate	28 days	++	+	0	0	0	0
	35 days	++	+	+	+	0	0
	42 days	++	++	++	+	0	0
Cordate	28 days	0	0	0	0	0	0
	35 days	++	+	+	0	0	0
	42 days	++	++	+	+	0	0

Compared with other plant species reported for phytoremediation of Cr (VI)-contaminated soil, *Pteris vittata* took up and accumulated significant amounts of Cr (up to 1,145 mg/kg in shoots and 5,717 mg/kg in roots) and did not die immediately from phytotoxicity. Our study suggests that Chinese brake fern is a potential candidate for phytoremediation of Cr (VI) -contaminated soils, even though plants showed severe phytotoxic symptoms at higher soil Cr concentrations. It can sustain in 10 to 20 ppm of

chromium concentration with some morphological and anatomical changes. Phytoremediation of As contaminated farmland using *P. vittata* has been performed in Chenzhou City, southern China since 2001 [19]. Recent studies with *P. vittata* showed that higher phosphate concentrations inhibited As (V) uptake in hydroponic culture [20], but enhanced As uptake in soil culture [21]. The amount of arsenic in the plants also correlated positively with the amount of phosphate in tissues and cells [22].

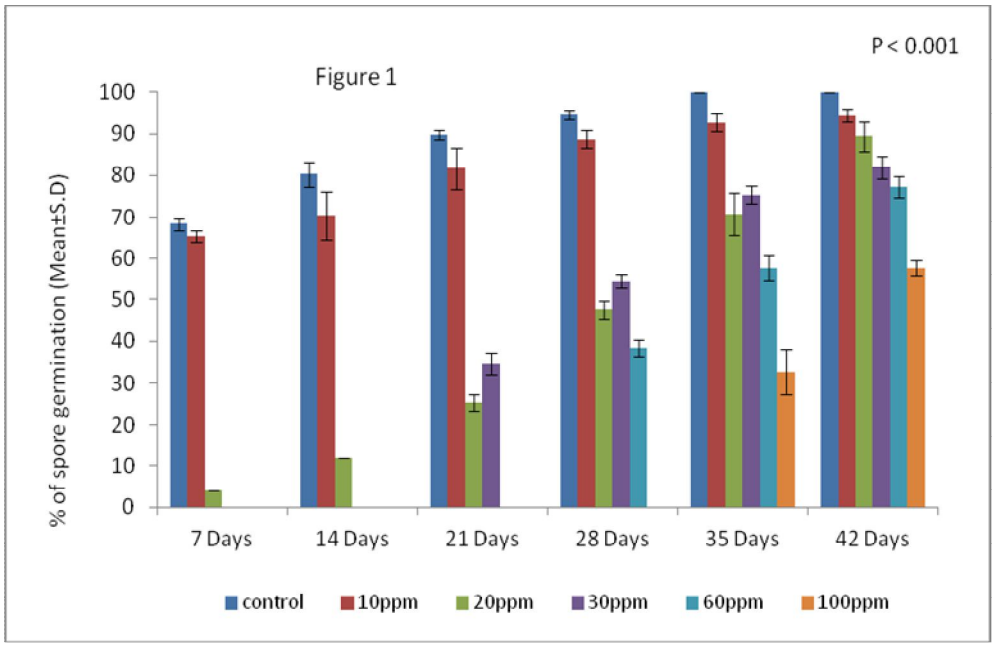

Figure-1 Effects of different chromium concentrations in spore germination of *P.vittata* were calculated. Values are means ±SE (n = 3). Values of different bars indicate that there is a significant difference, at P < 0.001, between various levels of Cr treatments.

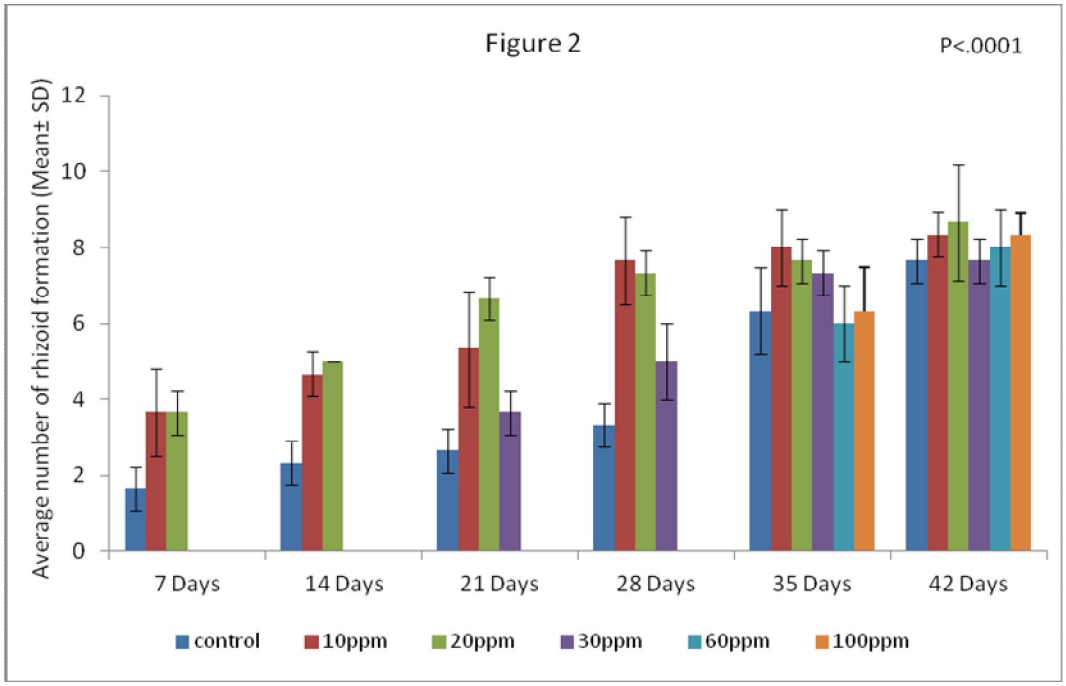

Figure-2 Effects of different chromium concentrations in rhizoid formation of *P.vittata* were calculated. Values are means ±SE (n = 3). Values of different bars indicate that there is a significant difference, at P < 0.0001, between various levels of Cr treatments.

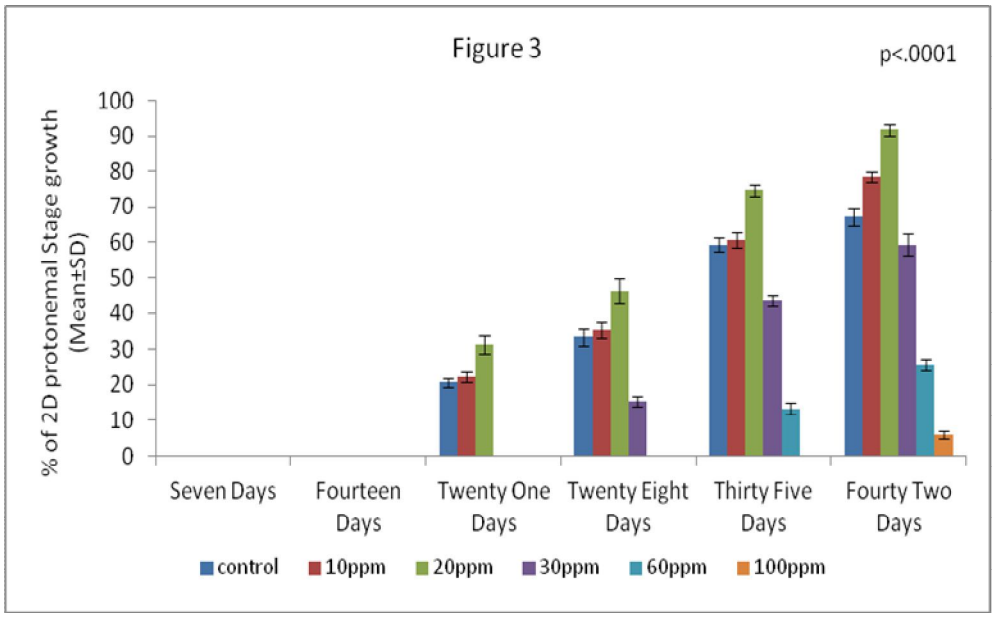

Figure-3 Percentage of protonemal cell growth of *Pteris vittata* in different treated concentration of chromium. The *p-value* was less than 0.0001. Percentages of 2D protonemal cell were calculated in each week after sowing. The error bars were standard error of the means from three replicates.

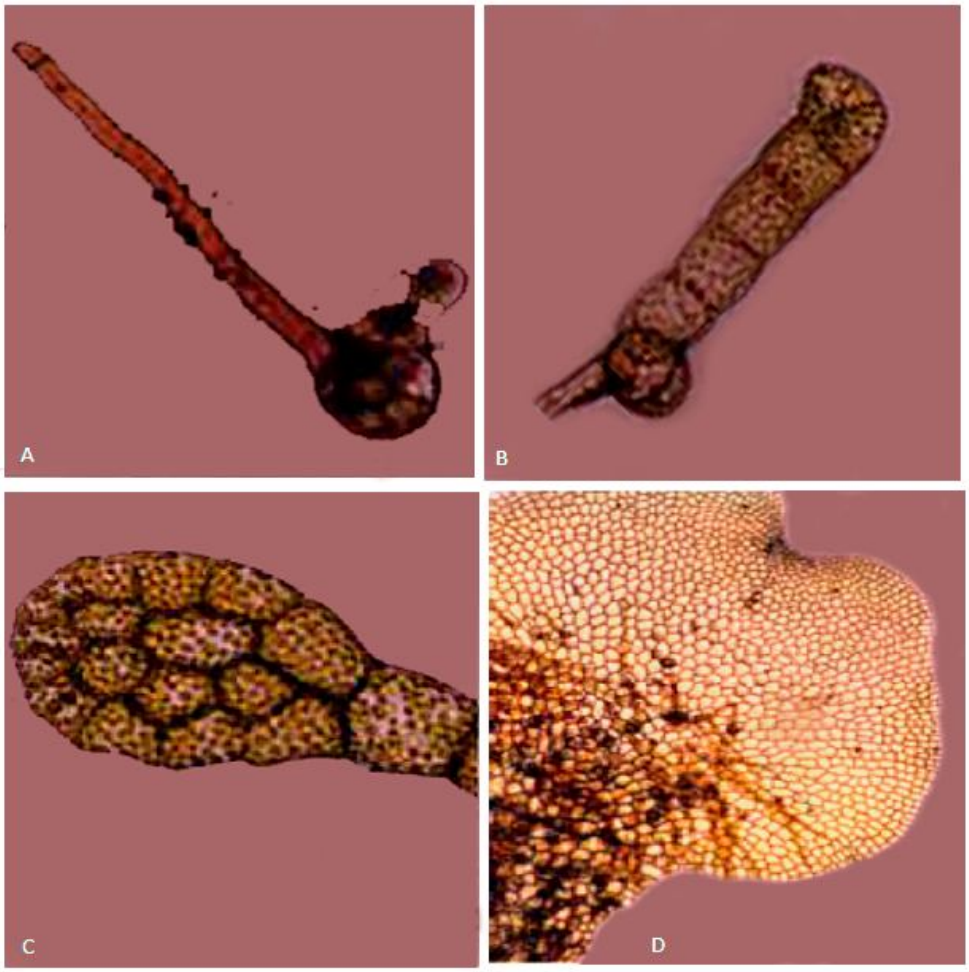

Figure 4- *Pteris vittata* propagation through tissue culture. A) Spore germination with rhizoid formation, B) Protonema cell formation with filamentous growth, C) Spatulate stage of the species, D) The multicellular spatulate stage leads to formation of Cordate gametophyte.

CONCLUSION

In vitro culture and subsequent regeneration of sporophytes from the spore culture of ferns will be very much helpful for mass cultivation as well as screening of phytochemicals present in the plant. The *Pteris vittata* able to sustain in chromium contaminants, it can be used as biosensors of subsurface contamination, thereby allowing investigators to quickly delineate contaminant plumes.

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REFERENCES

1. Cervantes C, Garcia JC, Devars S, Corona FG, and Tavera HL, Torres-guzman J Carlos, et al. (2001). "Interactions of chromium with micro-organisms and plants". *FEMS Microbiol Rev*. 25:335-347.
2. Wallace A, Soufi SM, Cha JW, and Romney EM. (1976)."Some effects of chromium toxicity on bush bean plants grown in soil". *Plant Soil*. 44: 471-473.
3. Shanker AK, Cervantes C, Loza-Tavera H, and Avudainayagam S. (2005). "Chromium toxicity in plants". *Environment International.* 31: 739-753.
4. Becquer T, Quantin C, Sicot M, and Boudot JP. (2003). "Chromium availability in ultramafic soils from New Caledonia". *Sci Total Environ*. 301: 251-61.
5. Nriagu JO.(1988). "Production and uses of chromium. Chromium in natural and human environment". *New York, USA7 John Wiley and Sons*. 81-105.
6. Barnhart J. (1997). "Occurrences, uses, and properties of chromium". *Regul Toxicol Pharm* 1997; 26:S3-7.
7. Kotas J. and Stasicka Z. (2000). "Commentary: chromium occurrence in the environment and methods of its speciation". *Environ Pollut*. 107:263-83.
8. Sen AK, Mondal NG, and Mandal S. (1994). "Toxic effects of chromium (VI) on the plant Salvinia natans L". *Environ Ecol*. 12:279-83.
9. Gupta K, Mehta R, Kumar N, and Dahiya DS. (2000). "Effect of chromium (VI) on phosphorus fractions in developing sunflower seeds (Helianthus annuus L)". *Crop Res*. 20:46-51.
10. Nichols PB, Couch JD, and Al Hamdani SH. (2000). "Selected physiological responses of *Salvinia minima* to different chromium concentrations". *Aquat Bot.* 68:313-9.
11. Ma L Q, Komar K M, and Tu C et al. (2001). "A fern that hyperaccumulates arsenic". *Nature*. 409: 579.
12. Slonecker, E.T., Haack, B.N. and Price, S.D. (2009). Spectroscopic Analysis of Arsenic Uptake in *Pteris* Ferns. *Remote Sensing*, 1(4), 644-675.
13. An, Z. Z., Z. C. Huang, et al. (2006). "Zinc tolerance and accumulation in *Pteris vittata* L. and its potential for phytoremediation of Zn- and As-contaminated soil." *Chemosphere.* 62(5): 796-802.
14. Kertulis-Tartar, G. M., L. Q. Ma, et al.(2006). "Phytoremediation of an arsenic-contaminated site using *Pteris vittata* L.: a two-year study." *Int J Phytoremediation.* 8(4): 311-22.
15. Natarajan, S., R. H. Stamps, et al. (2008). "Phytofiltration of arsenic-contaminated groundwater using *Pteris vittata* L.: effect of plant density and nitrogen and phosphorus levels." *Int J Phytoremediation;* 10(3): 220-33.
16. Shukla S. P. and Khare P.B. (2012). *In-vitro* shoot regeneration via caulogenesis in fern, *Pteris vittata* L. *Journal of environmental biology.* 33 (4).
17. Ozaki T, Enomoto S, Minai Y, Ambe S and Makide Y. (2000). "A survey of trace elements in pteridophytes". *Biological trace element research.* 74:259-273.
18. Korn RW. (1974). Computer simulation of the early development of the gametophyte of Dryopteris thelypteris (L.) Gray. Botanical Journal of the Linnean Society. 68: 163-171.
19. Liao X Y, Xiao X Y, and Chen T B. (2003). Effects of Ca and As addition on As, P and Ca uptake by hyperaccumulator *Pteris vittata* L. under sand culture". *Acta Ecol Sinica.* 23(10): 2058-2065.
20. Tu S, Ma L Q, and MacDonald G E et al. (2004)."Effects of arsenic species and phosphorus on arsenic absorption, arsenate reduction and thiol formation in excised parts of *Pteris vittata* L.". *Environ Exp Bot*. 51: 121-131.
21. Cao X, Ma L Q, and Shiralipour A. (2003). "Effects of compost and phosphate amendments on arsenic mobility in soils and arsenic uptake by the hyperaccumulator *Pteris vittata* L.". *Environ Pollut*. 2003; 126: 157-167.
22. Chen T B, Fan Z L, and Lei M et al. (2002a). "Effect of phosphorus on arsenic accumulation in As-hyperaccumulator *Pteris vittata* L. and its implication". *Chin Sci Bull*. 47(22): 1876-1879.