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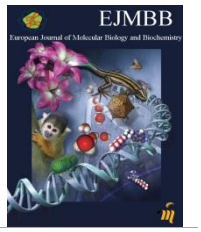

SDS – PAGE PROTEIN PROFILE OF *BOLBITIS SEMICORDATA* (MOORE) CHING AND *BOLBITIS APPENDICULATA* VAR. *ASPLENIFOLIA* (BORY) SLEDGE FROM SOUTH INDIA

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ABSTRACT

The present study was aimed to know the inter-specific variation between the two species viz., *Bolbitis semicordata* (Moore) Ching and *Bolbitis appendiculata* var. *asplenifolia* (Bory) Sledge using SDS-PAGE. 500 mg of sporophytes were ground in an ice cold mortar and pestle with 500µl 0.1M phosphate buffer pH 7.0. The resultant slurry was centrifuged at 10,000 rpm for 10 min at 4ºC in cooling centrifuge and the supernatant was stored at 4ºC before use. For separtion of proteins, SDS-PAGE was carried out. A total of 12 bands with multiple regions of activity with varied MW-Rf values were observed in the protein electrophoretic system of *Bolbitis* species viz., *Bolbitis semicordata* and *Bolbitis appendiculata* var. *asplenifolia*. The SDS-PAGE gel system of *Bolbitis* failed to show the similarity betweent the studied two species *Bolbitis semicordata* and *Bolbitis appendiculata* var. *asplenifolia*. These protein profiles can be used as a biochemical marker to identify the studied two *Bolbitis* species.

INTRODUCTION

Due to the advancement in the analytical chemistry and molecular biology, the application of biochemical constitutents and molecular data to find the solution for the taxonommical problems has significantly increased in recent years, and nowadays, chemotaxonomy is generally considered as an accepted and established discipline in the plast systemtics [1]. Plant molecular systematics and chemosystematics studies out comes confirmed the application of chemical data and molecular data in plant taxonomic problem resolution at various hierarchical levels [2]. In the last three decades the biochemical and molecular especially the DNA analysis has vastly expanded and studies employing the biochemical and DNA to infer the phylogeneis. At global number of studies were focused on the biochemical and molecular systetmatics of pteridophytes. With refernce to India very few studies were undertaken [3-5]. Roussel [6] enumerated the ethnobotanical usage of *Bolbitis pergamentacea* (Maxon) Ching as anti-inflammatory and immunomodulatory agent by the community of Q'eqchi' Maya Healers Association (QMHA) of Belize, and the Cree of Eeyou Istchee (CEI) of northern Quebec. Mathew and Tensy Issac [7] studied the ontogeny and vasculature of viviparous buds of *Bolbitis semicordata* (Baker) Ching. They observed the bud on superficial origin, later developing in to a rhizomatous structure. The bud developed root primordia from a meristematic cell having four cutting faces. They failed to observe sexual reproduction during viviparous germination. Mazumder *et al*., [8] studied the effect of different growth regulators on different characters viz. germination percentage, fresh and dry weight of prothallus and sporophytes of *Bolbitis costata*. Molecules based knowledge concerning the biochemical and molecular variation and taxonomic relatioship in the India pteridophytes are limited. A few

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studies are carried out on the isoperoxidase analysis on south Indian pteridophytes [9-12]. For the identification of pteridophytes, the biologist are totally depends on the morphological and cytological characters to determine the phylogenetic relationships. With reference to Indian context, there is no report on the phytochemical and molecular studies on *Bolbitis* species. To fulfill the lacuna as fisrt step, in the present study inter-specific variation between the two species viz., *Bolbitis semicordata* (Moore) Ching and *Bolbitis appendiculata* var. *asplenifolia* (Bory) Sledge was carriedout.

MATERIALS AND METHODS

The young sporophytes of *Bolbitis semicordata* (Moore) Ching and *Bolbitis appendiculata* var. *asplenifolia* (Bory) Sledge were collected from the natural habitats. For protein isolation 500 mg of sporophytes were ground in an ice cold mortar and pestle with 500µl 0.1M phosphate buffer pH 7.0. The resultant slurry was centrifuged at 10,000 rpm for 10 min at 4ºC in cooling centrifuge and the supernatant was stored at 4ºC before use. For separtion of proteins, SDS-PAGE was carried out. After electrophoresis the gel was stained with coomasie brilliant blue for 3 hours and the stained gel was destained with destainer I and II then fixed with fixative. The stained gel was scored using a Vilber Loubermat gel documentation system and banding profiles of protein was compared by zymogram.

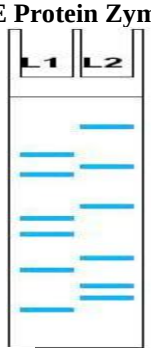
RESULTS

The SDS-PAGE protein profile of two *Bolbitis* species were demonstrated in Table – 1 (Fig. 1). A total of 12 bands with multiple regions of activity with varied MW-Rf values were observed in the protein electrophoretic system of *Bolbitis* species viz., *Bolbitis semicordata* and *Bolbitis appendiculata* var. *asplenifolia*. The SDS-PAGE gel system of *Bolbitis* failed to show the similarity between the studied two species *Bolbitis semicordata* and *Bolbitis appendiculata* var. *asplenifolia*. The proteins with MW-Rf values viz., 0.271, 0.304, 0.445, 0.489, 0.619 and 0.750 were showed their restricted occurrence in *Bolbitis semicordata*. Similarly *Bolbitis appendiculata* var. *asplenifolia* also demonstrated their uniqueness by the expression of proteins with MW-RF viz., 0.188, 0.294, 0.423, 0.576, 0.682 and 0.717. The results obtained through the electrophoretic separation clearly indicated the *inter*-specific variation between the studied *Bolbitis* species viz., *Bolbitis semicordata* and *Bolbitis appendiculata* var. *asplenifolia*. These protein profiles clearly distinguished the morphologically similar *Bolbitis* species viz., *Bolbitis semicordata* and *Bolbitis appendiculata* var. *asplenifolia*. These protein profiles can be used as a biochemical marker to identify the studied two *Bolbitis* species.

Table 1. SDS-PAGE Protein pattern of *Bolbitis semicordata* (Moore) Ching and *Bolbitis appendiculata* var. *asplenifolia* (Bory) Sledge

MW- Rf-	*Bolbitis semicordata*	*Bolbitis appendiculata* var. *asplenifolia*
0.188	-	+
0.271	+	-
0.294	-	+
0.304	+	-
0.423	-	+
0.445	+	-
0.489	+	-
0.576	-	+
0.619	+	-
0.682	-	+
0.717	-	+
0.750	+	-

Fig 1. SDS-PAGE Protein Zymogram of *Bolbitis*



L1 - *Bolbitis semicordata*; L2 - *Bolbitis appendiculata* var. *asplenifolia*

DISCUSSION

Characterization and quantification of the genetic diversity and information on the genetic diversity within and among closely related species is essential for a rational use of plant genetic resources. Inter specific and intra specific variation among the species is valuable one for analyzing the germplasm in breeding programmes [13]. The protein profiling of germplasm and use of genetic markers have been widely and effectively used to determine the taxonomic and evolutionary aspects of several crops and speceis [14-16]. Morphological characterization is the first step in the classification and description of any germplasm [17-18]. In the modern taxonomy, electrophoresis supplements information to classical taxonomy and should not be dissociated from morphological, anatomical and cytological observations. Characterization of germplasm using protein profile has received special attention among the plant biologists. Electrophoretic techniques are used on large scales in protein and enzyme analysis to identify and characterize the genotype differences among plant species and varieties. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is economically cheap and extensively used biochemical technique for analysis of genetic structure of germplasm [19]. In the present study also, the SDS-PAGE is employed to distinguish the variation between the two ferns viz., *Bolbitis semicordata* and *Bolbitis appendiculata* var. *asplenifolia*. The results of the present observations were supplemented with the previous observation [19]. The results of the present study provided the species specific idenitfication biochemical markers for the ferns *Bolbitis semicordata* and *Bolbitis appendiculata* var. *asplenifolia*. The results of present study clearly explained the relatioships between the studied two species on the basis of banding distribution in the gel system. The distinctiveness of *Bolbitis semicordata* and *Bolbitis appendiculata* var. *asplenifolia* was confirmed by the protein polymorphism and morphological charactes. The protein marker will very much be useful in future research dealing with pharmaceuticals and molecular plant systematics of Indian pteridophytes. Several researchers have confirmed the usefulness of different SDS-PAGE proedures in plant taxonomic, evolutionary and genetic relationship studies [14-16]. The results of the present study also supplmented the previous observations. The results of present study clearly explained the relatioships between the studied two speceis and distinguished based on the protein banding pattern in the gel system.

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