IJREB

ISSN **2321-743X**

International Journal of Research in

Engineering and Bioscience

Volume **3** Issue **5** (Pages 36-41)

Journal home page: www.ijreb.org

PROTEOMIC EXPRESSION STUDIES ON GAMETOPHYTES WITH JUVENILE SPOROPHYTES AND MATURED SPOROPHYTES OF SELECTED PTERIDOPHYTES FROM GORAKHPUR

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ABSTRACT

The present study was aimed to pinpoint the protein expression between the gametophytes with juvenile sporophytes and matured sporophytes of *Adiantum lunulatum* Burm., *Lygodium flexuosum* (L.) Sw. and *Adiantum peruvianum* Koltz. from Gorakhpur. To reveal the proteomic variation between the gametophyte with juvenile sporophytes and matured sporophytes of *A. lunulatum*, *L. flexuosum* and *A. pervianum*, SDS-PAGE was carried out to obtained protein bands following the method described by Anbalagan. The virtual protein positions of gametophyte with juvenile sporophytes and matured sporophytes of *A. lunulatum*, *L. flexuosum* and *A. pervianum* were revealed by SDS-PAGE. The protein profile of the selected pteridophytes showed the difference of protein expression at different developmental stages. Thus in conclusion, there is indication for the functional difference between different stages of life cycle of the fern, particularly in the gametophyte - spootphytes.

KEYWORDS: Pteridophytes; Proteomics; Gametophytes; Sporophytes

INTRODUCTION

Next to angiosperm, pteridophytes are the most important component of the flora of this major region of species-diversity in number. India is particularly rich in pteridophytic flora with 1250 species of ferns belonging to 70 families and 191 genera. Among them, 233 species of ferns occur in South India (Manickam and Irudayaraj, 1992; Chandra *et al*., 2008). Though the study of ferns has been fundamental to our understanding of vascular plant biology, even

the most basic aspects of fern developmental and reproductive biology have only begun to be examined. Ferns have both independent gametophytes and sporophytes; can reveal important aspects of the morphogenesis of organisms with two freeliving and fundamentally different generations. With regard to gametophytes of pteridophytes, morphological studies are mostly completed through in vitro spore cultures (Manickam *et al*., 2003; Johnson and Manickam, 2006; Johnson *et al*., 2008; Johnson and Manickam, 2009; Johnson and Manickam, 2012; Johnson and Manickam, 2015; Johnson and Manickam, 2015a). There are only sporadic reports on the morphology, developmental and reproductive biology from wild. Michael *et al* 16 have clearly explained difference between laboratory and field populations of fern gametophytes. Ranker and Houston17 compared field-collected data to the laboratory-based data of the cultured gametophytes of the Hawaiian endemics *Sadleria cyatheoides* and *S. pallida* grown on mineral-enriched. They observed that natural field studies are better than laboratory conditions in general, for fern gametophyte sexual development. Revathy *et al.,* (2011) studied the reproductive biology and protein expression studies on the different developmental stages of gametophytes and sporophytes of *Adiantum raddianum* Presl (Adiantaceae), *Arachniodes tripinnata* (Goldm.) Sledge and *Dryopteris sparsa* (Buch. Hm. Ex. D. Don) C. Chr belonging to the family Dryopteridaceae and *Odontosoria chinensis* (L.) J. Smith (Lindsaeaceae) collected from Kothayar, Tirunelveli Hills (Western Ghats, South India). Sivaraman *et al.,* (2011) also studied the reproductive biology and protein expression studies on the different developmental stages of gametophytes and sporophytes of some selected ferns viz., *Tectaria paradoxa* (Fee) Sledge, *Araiostegia hymenophylloides* (Bl.) Copel and *Deparia petersenii* (Kunze) M. Kato. Fresh. Buth there is no report on the biochemical varaition between the youg and mature sporophytes of the pteridophytes. With this knowledge the present study was aimed to pinpoint the protein expression between the gametophytes with juvenile sporophytes and matured sporophytes of *Adiantum lunulatum*, *Lygodium flexuosum* and *Adiantum pervianum* from Gorakhpur.

MATERIALS AND METHODS

Collection of plant materials fresh and healthy explants viz., gametophyte with juvenile sporophytes and matured sporophytes were collected in and around Kusmi forest, Gorakhpur, Uttar pradesh India. Regular observation was made in the field to observe the development of both gametophytic and sporophytic generation of selected ferns during December 2014 to February 2014. The selected specimens were identified based on "Pteridophytic Flora of the Western Ghats, South India" by Manickam and Irudayaraj (1992). The provenances of the specimens are viz., *Adiantum lunulatum* (SAC – 308 and SAC – 310 Gametophytes with juvenile sporophytes

and Matured sporophytes respectively), *Lygodium flexuosum* (SAC – 311 and SAC – 313 Gametophytes with juvenile sporophytes and Matured sporophytes respectively) and *Adiantum pervianum* (SAC – 320 and 321 Gametophytes with juvenile sporophytes and Matured sporophytes respectively.

Protein isolation and separation

Protein isolation and separation was carried out by the standard method described by Anbalagan (1999). The fresh gametophyte with juvenile sporophytes and matured sporophytes of selected ferns were harvested and washed in deionised water. The explants were mashed in a pre-chilled mortar with 500µL of phosphate buffer (pH 7.0). The resultant slurry was centrifuged at 10000 rpm for 10 min at 40C in a Micro 22 R centrifuge and the supernatant was collected and stored at 4°C before use. SDS-PAGE was carried out at 25°C in the air conditioned room. Separation of protein was carried out at 50V till the tracking dye reaches the separating gel and at 100V thereafter for 3-5 hours or until the tracking dye had migrated to the bottom of the gel. After electrophoresis, the gels were carefully removed from the mold and stained.

RESULTS AND DISCUSSIONS

The relative positions of the protein bands of gametophytes with juvenile sporophytes and matured sporophytes of *Adiantum lunulatum* revealed by SDS-PAGE showed obvious changes in the banding profiles (Table 4; Fig. 1 A and B). The protein bands are showed between the MW-Rf ranges from 0.21 to 0.97. The MW-Rf values 0.21 and 0.84 dispalyed their expression only with gametophyte with juvenile sporophytes. The MWRf 0.68, 0.81, 0.89 and 0.97 confirmed their commonly occurence in the gametophyte with juvenile sporophytes and matured sporophytes. The proteins with Rf values 0.35, 0.39, 0.48, 0.52, 0.56, 0.61, 0.66, 0.76 and 0.94 showed their restricted existence in matured sproophytes of *A. lunulatum*.

The virtual protein positions of *Lygodium flexuosum* gametophytes with juvenile sporophytes and matured sporophytes were revealed by SDS-PAGE dispalyed the various pattern of proteins in the gel system (Table 1; Fig. 1 A and B). The protein bands are appeared between the MW-Rf ranging from 0.48 to 0.95. The MW-Rf values 0.48, 0.52, 0.56, 0.61, 0.66, 0.7, 0.73, 0.84, 0.87 and 0.95 restricted their expression only in mature sporophytes. The MWRf values 0.68 and 0.71 showed their limited presence only in gametophyte with juvenile sporophytes. The protein with Rf value 0.81 showed its commonly occurence in gametophyte with juvenile sporophytes and matured sporophytes of *L. flexuosum*.

The protein banding positions of the gametphytes with juvenile sprophytes and matured sporophytes of *Adiantum pervianum* revealed by SDS-PAGE illustrated obvious changes in the banding profiles (Table 1; Fig. 1 A and B). The protein bands are demonstrated

between the MW-Rf values ranging from 0.58 to 0.87. The protein with MW-Rf values 0.58 and0.68 limited their expression only in matured sporophytes of *A. pervianum*. The protein with MW-Rf 0.66 is present only in gametophytes with juvenile sporophytes. The protein with MW-Rf 0.76 and 0.87 showed their commonly presence gametophytes with juvenile sporophytes and matured sporophytes of *A. pervianum*. The protein profile of the selected pteridophytes showed the difference of protein expression at different developmental stages. The proteins 0.81 showed its occurrence in the matured sporophytes of *A. lunulatum* and *L. flexuosum*. Similar to the present study the electrophoretic analysis of proteins of *Vittaria lineata* sporophytes and independently growing gametophytes of *Vittaria* indicated considerable differences in protein profiles (Farrar 1985). Similar to the present investigation Revathy *et al.*, (2011) and Sivaraman *et al.*, (2011) also observed the protein expression variation in the different developmental stages of pteridophytes. The genetic diversity of mature sporophytes of different ferns is well correlated with ploidy level of the species. When the number of bands in gametophytes with juvenile sporophytes and matured sporophytes is compared and analysed, surprisingly there are several (4-9) new bands appeared in matured sporophytes than the gameophytes with juvenile sporophytes. It may be due to the synthesis of several kinds of proteins/enzymes during the shifting / transition stage of life cycle from gametophytic phase to diploid sporophytic phase. Thus in conclusion, there is indication for the functional difference between different stages of life cycle of the fern, particularly in the gametophyte - spootphytes. From the present study, it is also clear that ferns are good materials not only for morphogenetic studies, but also for studying the genetic control of different stages of life cycle, due to the presence of independent sporophytic and gametophytic generations.

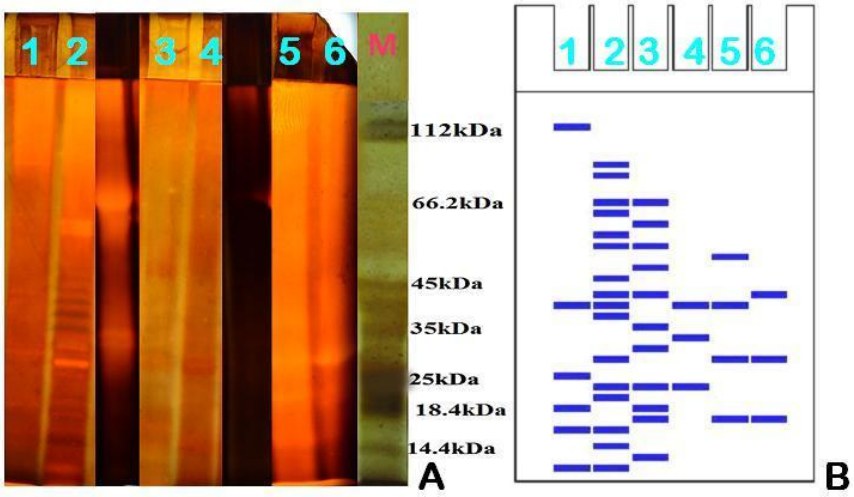

Fig. 1: SDS – PAGE Profile and Zymogram of Gametophytes with Juvenile Sporophytes And Matrure Sporophytes of Selected Pteridophytes

A and B: 1 - *A. lunulatum* Gametophytes with juvenile sporophytes; 2 - *A. lunulatum* Matured sporophytes; 3 - *L. flexuosum* Gametophytes with juvenile sporophytes; 4 - *L. flexuosum* Matured sporophytes; 5 - *A. pervianum* Matured sporophytes and 6 - *A. pervianum* Gametophytes with juvenile sporophytes

Table 1: Protein Expression of gametophytes with Juvenile sporophytes and Matrure sporophytes of selected Pteridophytes

MW-Rf	**Positions**	*A. lunulatum* Gametophytes with juvenile sporophytes	*A. lunulatum* Matured Sporophytes	*L. flexuosum* Matured Sporophytes	*L.flexuosum* Gametophytes with juvenile sporophytes	*A. pervianum* Matured Sporophytes	*A. pervianum* Gametophytes with juvenile sporophytes
0.21	**PP3[1]**	*					
0.35	**PP4[1]**		*				
0.39	**PP4[2]**		*				
0.48	**PP5[1]**		*	*			
0.52	**PP6[1]**		*	*			
0.56	**PP6[2]**		*	*			
0.58	**PP6[3]**					*	
0.61	**PP7[1]**		*	*			
0.66	**PP7[2]**		*	*			*
0.68	**PP7[3]**	*	*		*	*	
0.7	**PP7[4]**			*			
0.71	**PP8[1]**				*		
0.73	**PP8[2]**			*			
0.76	**PP8[3]**		*			*	*
0.81	**PP9[1]**	*	*	*	*		
0.84	**PP9[2]**	*		*			
0.87	**PP9[3]**			*		*	*
0.89	**PP9[4]**	*	*				
0.94	**PP10[1]**		*				
0.95	**PP10[2]**			*			
0.97	**PP10[3]**	*	*				

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