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Inter-Specific Variation Studies among *Nephrolepis* using SDS-PAGE

Johnson M*

Centre for Plant Biotechnology, Department of Botany, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India

Abstract

The present study was aimed to reveal the biochemical similarity and varaition among the three taxonomically confused species *Nephrolepis exaltata* (L.) Schott, *Nephrolepis auriculata* (L.) Trimen and *Nephrolepis multiflora* (Roxb.) Jarret using SDS-PAGE analysis. To reveal the inter-speciifc variation among the selected *Nephrolepis*, SDS-PAGE was carried out to obtain protein bands following the method described by Anbalagan. Multiple regions (8) of activity were observed from protein electrophoretic system of *Nephrolepis*. A total of 19 bands with various Rf values and molecular weight were demonstrated in the SDS-PAGE gel system of *Nephrolepis*. Among the three species of *Nephrolepis*, *N. exaltata* showed maximum number of protein bands (9) followed by *N. multiflora* (6) and *N. auriculata* (4). Each region expressed different proteins which act as representative of the expression of a particular gene in the studied species of *Nephrolepis*. The similarity indices were calculated and cladogram was constructed based on the protein profiles of *Nephrolepis* and revealed the similarities and variation among the studied *Nephrolepis* species. The results obtained in this work also showed that SDS-PAGE analysis can provide an easy, low cost and quick way for the identification of inter-specific variation among the selected *Nephrolepis* species. SDS-PAGE analysis provided strong basis for the discrimination of genotypes on the basis of specific polypeptide fragments.

Keywords: *Nephrolepis;* Protein profile; SDS-PAGE

Introduction

Nephrolepis is a tropical genus with 40 species and is identified worldwide (Friedrich 2005). In India nearly eight species are enumerated [1]. Among the eight speceis, except *Nephrolepis delicatula* all other species are recorded in South India. But Manicakam and Irudayaraj [2] noted only two indigenous *Nephrolepis* species, other species nomenclature, identity and distribution has to be confirmed. Similarly Sledge [3] also pointed out the nomenclatural problem of *Nephrolepis cordifolia*, *Nephrolepis auriculata* and *Polystichum auriculatum*. Mickel and Beitel [4] also noted the misidentification of *Nephrolepis exaltata* and *Nephrolepis biserrata*. *Nephrolepis multiflora* (Roxb.) Jarret is distinguished morphologically by the charactereous frond and finely granulose exine. *Nephrolepis auriculata* (L.) Trimen is identified morphoogically based on the herbaceous fronds, exine with reticulate ridges and tubers on the roots. *Nephrolepis exaltata* (L.) Schott, with alternate pinnae (the small "leaflets" on either side of the midrib), each pinna being 2-8 cm long. The pinnae of *N. exaltata,* are generally deltoid and the edges appear slightly serrate. Similar to *N. multiflora* the *N. exaltata* also not possesed the tubers in the roots. *Nephrolepis exaltata* (L.) Schott, *Nephrolepis auriculata* (L.) Trimen and *Nephrolepis multiflora* (Roxb.) Jarret are morphologically similar and they possess taxonomic problems especially in their identification. Much more studies therefore, need to be carried out to provide taxonomic features that will delimit the species. Polyacrylamide Gel Electrophoresis (PAGE) is a versatile biochemical technique to detect genetic variation. In recent years, there has been explosion in the availability of different types of genetic markers [5]. With this knowledge the study was aimed to reveal the biochemical similarity and variation among the three taxonomically confused species *Nephrolepis exaltata* (L.) Schott, *Nephrolepis auriculata* (L.) Trimen and *Nephrolepis multiflora* (Roxb.) Jarret using SDS-PAGE anaysis.

Materials and Method

For the electrophoresis studies, 500 mg of *Nephrolepis exaltata* (L.) Schott, *Nephrolepis auriculata* (L.) Trimen and *Nephrolepis multiflora* (Roxb.) Jarret young individual croziers were harvested from Kodaikannal Botanic Garden, Eettipallum, Kodaikannal and ground on ice cold mortar and pestle with 0.1 M phosphate buffer (pH 7.0). The resultant slurry was centrifuged at 10,000 rpm for 10 min at 4°C in cooling centrifuge and the supernatant was stored at -70°C before use. SDS-PAGE was carried out to obtain protein bands following the method described by Anbalagan [6]. After electrophoresis the gel was observed using a Vilber Loubermat gel documentation system and banding profiles of protein was compared by zymogram. For the inter-specific relationship studies, the protein profile was converted into a "1" and "0" matrix, to indicate the presence or absence of the Rf Values, respectively. Genetic similarities (GS) were estimated according to Nei and Li [7]. To demonstrate the inter-specific relationship among the studied *Nephrolepis*, a dendrogram was constructed by UPGMA using NTSYSpc-2.0 software.

Results and Discussion

The relative positions of the protein bands of the studied *Nephrolepis* species viz., *Nephrolepis exaltata* (L.) Schott, *Nephrolepis auriculata* (L.) Trimen and *Nephrolepis multiflora* (Roxb.) Jarret collected from various localities of South India were revealed by SDS-PAGE. Multiple regions (8) of activity were observed from protein electrophoretic system of *Nephrolepis*. A total of 19 bands with various Rf values and molecular weight were demonstrated in the SDS-PAGE gel system of *Nephrolepis* (Table 1; Figure 1). Among the three species of *Nephrolepis*, *N. exaltata* showed maximum number of protein bands (9) followed by *N. multiflora* (6) and *N. auriculata* (4). The observed protein profile demonstrated the role of protein in similarity and variation between the studied species of *Nephrolepis*. Each region expressed different proteins which act as representative of the expression of a particular gene in the studied species of *Nephrolepis*. Based on the occurrence of proteins in the *Nephrolepis* gel system, the protein profiles were classified into

***Corresponding author:** Marimuthu Johnson, Centre for Plant Biotechnology, Department of Botany, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India, Tel: +979786924334; Fax: +914622561765; E-mail: ptcjohnson@gmail.com

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MW - Rf	Mol. Wt. in K Da	Region	Position	*N. multiflora*	*N. auriculata*	*N. exaltata*
0.08	177.8	1	PP1[1]	+		+
0.10	162.2	2	PP2[1]			+
0.16	123.0		PP2[2]			+
0.18	100.0		PP2[3]	+		+
0.20	99.5	3	PP3[1]		+	
0.22	96.2		PP3[2]	+		
0.24	93.3		PP3[3]			+
0.29	87.0		PP3[4]			+
0.31	80.3	4	PP4[1]		+	
0.35	61.6		PP4[2]			+
0.37	58.5		PP4[3]		+	+
0.43	50.1	5	PP5[1]	+		
0.47	46.1		PP5[2]			+
0.55	39.2	6	PP6[1]	+		
0.65	29.6	7	PP7[1]	+		
0.72	25.1	8	PP8[1]		+	

Table 1: Protein profile of *Nephrolepis* species in SDS-PAGE gel system.

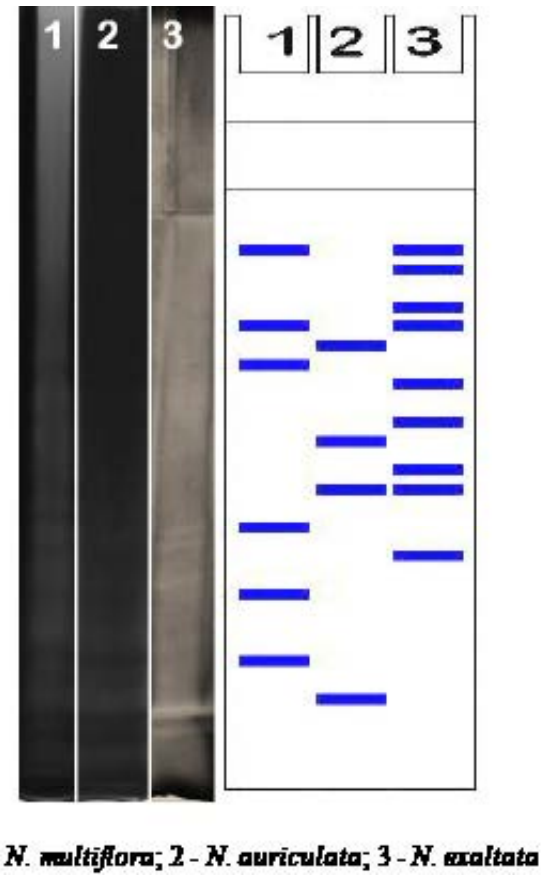

Figure 1: SDS PAGE pattern and zymogram of SDS-PAGE protein profile of *Nephrolepis*.

ten regions. Region 1 observed with only one protein (PP1[1]) with Rf-0.02 and MW- 177.8 KDa showed its presence in *N. exaltata* and *N. multiflora*. Region 1 explained the similarity between the *N. exaltata* and *N. multiflora*. Region 2 showed four protein bands with three positions (PP2[1-3]). PP2[1] (Rf-0.10; MW-162.2 KDa) and PP2[2] (Rf-0.16; MW-123.0 KDa) was displayed their existence only in *N. exaltata*. PP2[3] (Rf-0.18; MW-100.0 kDa) represented its common occurrence in *N. exaltata* and *N. multiflora*. Two unique bands were found in region 2 and explained the uniqueness of *N. exaltata*. Region 3 depicted four bands with four positions. PP3[1] (Rf-0.2; MW-99.5 KDa) expressed its unique occurrence in *N. auriculata*. Similarly PP3[2] (Rf-0.22; MW-96.2 KDa) was present only in *N. multiflora*. PP3[3] (Rf-0.24; MW- 93.3 KDa) and PP3[4] (Rf-0.29; MW-87.0 KDa) showed their existence only in *N. exaltata*. In region 3, the studied three species explained its exclusive character with the distinct Rf value and protein occurrence in the protein gel system of *Nephrolepis*. Region 4 represented four bands with three positions. PP4[1] (Rf-0.31; MW-80.3 KDa) was showed its occurrence only in *N. auriculata*. PP4[2] (Rf-0.35; MW-61.6 KDa) was demonstrated its unique existence only in *N. exaltata*. PP4[3] (Rf-0.37; MW-58.5 KDa) showed its common presence in *N. auriculata* and *N. exaltata*. *N. multiflora* failed to express its occurrence in this region. The region 4 clearly explained the biochemical similarities and difference between *N. auriculata* and *N. exaltata*. Region 5 depicted two bands with two positions only. PP5[1] (Rf-0.43; MW-50.1 KDa) was distinct to *N. multiflora* that explained its special occurrence in the region 5. Similarly PP5[2] (Rf-0.47; MW-46.1 KDa) was observed in *N. exaltata*. In this region *N. auriculata* failed to demonstrate its existence. The region 5 also clearly distinguished the difference among the studied species with specific protiens. Region 6 (PP6[1] - Rf-0.55; MW-39.2 KDa), 7 (PP7[1] - Rf-0.65; MW-29.6 KDa)) and 8 (PP8[1] - Rf-0.72; MW-25.1 KDa)) represented with only one proteins. The protein PP6[1] and PP7[1] showed their occurrence only in *N. multiflora*. PP8[1] (Rf-0.72; MW-25.1 KDa) displayed its unique presence in *N. auriculata* only which explained its variation with other studied species of *Nephrolepis*. Regions 9 and 10 were failed to express protein occurence in the studied *Nephrolepis* species. The banding patterns of proteins in the SDS-PAGE gel system of *Nephrolepis* discussed the similarity and variation between the studied three species of *Nephrolepis*. The similarity indices were calculated and cladogram was constructed based on the protein profiles of *Nephrolepis* and revealed the similarities between the *N. multiflora* and *N. exaltata*, the *N. auriculata* showed the variation and occupied a separate clade in the cladogram (Figure 2). The evolutionary tree which constructed based on the protein profile expressed two clusters (C_1 and C_2). The cluster (C_1) includes two species of *Nephrolepis* viz., *N. multiflora* and *N. exaltata*. The cluster (C_2) is simply with one species *N. auriculata*, the cladogram constructed based on the protein profile showed the closeness and divergence among the three *Nephrolepis* species. Among the various analytical tools, electrophoresis is a relatively simple, rapid and highly sensitive tool to study the properties of proteins and nucleic acids. Studies of protein variation are important tool (PAGE) that has often been employed to know the biochemical variation, inter and intra specific variation and evolutionary relationships among the plant species [8-13]. In addition, researchers employed the Poly Acrylamide Gel Electrophoresis (PAGE) to know information about the molecular weights and charges of proteins. Protein electrophoresis is a powerful tool for population genetics [14]. Ghafoor et al. [15] employed protein profiles as genetic markers to resolve taxonomic and evolutionary problems of *Cicer arietinum*. Harendra Singh et al. [16] differentiated *Rhizobium* inoculated Desi and Kabuli Chickpea (*Cicer arietinum* L.) genotypes using SDS-PAGE. Dudwadkar et al. [17] enumerated the protein diversity of Cucurbitaceae using SDS-PAGE protein profile

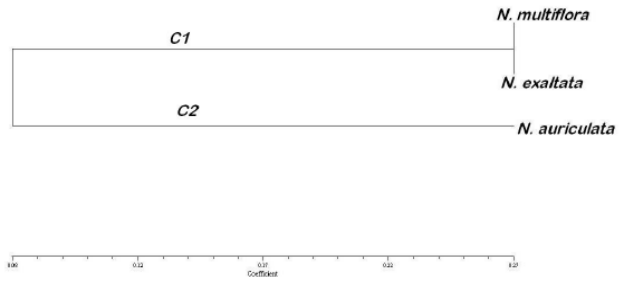

Figure 2: Cladogram based on the protein profile of *Nephrolepis*.

and found the similarities and variations among the Cucurbitaceae members. Sivaraman et al. [18] and Revathy et al. [19] revealed the protein expression on various morphogenetic developments of selected ferns from Western Ghats. Narayani and Johnson [20] studied inter specific proteomic studies on selected *Selaginella* species using SDS-PAGE. In the present study also SDS-PAGE protein profiles distinguished the similarity and variation among the three *Nephrolepis* species. The results of the present study also supplemented the previous observation and application by expressing inter-specific protein variation among the three *Nephrolepis* species. These banding profiles can be used as biochemical and pharmacognostical marker to distinguish the medicinally important *Nephrolepis* from its adulterants in the pharmaceutical industries. Characterization of genetic diversity in *Nephrolepis* species, offers an opportunity for breeders for its exploitation in wide hybridization programs in the horticulutre. Furthermore, the results obtained in this work also showed that SDS-PAGE analysis can provide an easy, low cost and quick way for the identification of inter-specific variation among the selected *Nephrolepis* species. SDS-PAGE analysis provided strong basis for the discrimination of genotypes on the basis of specific polypeptide fragments.

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