

SDS-PAGE Analysis of Leaf Galls of *Alstonia scholaris* (L.) R. Br.

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Abstract

Alstonia scholaris R. Br. is an elegant evergreen tree of family Apocynaceae, generally bears leaf galls caused by an insect *Pauropsylla tuberculata* Crawf. In the present investigation the electrophoretic protein analysis technique was used, which revealed that some of the protein bands are varied and shown their presence and absence in gel at different stages of gall formation. The amount of total protein increased during early development and young stages of gall formation and falls down in older stages. It is due to a rapid enzymatic activity in gall tissue during early and mature stages as a response to insect interaction. Pathogens inject some elicitors and lead to the synthesis of different type of enzymes and some specific proteins at high amount in the plant. The insect switches the defense mechanism of the plant which results increased amount of some specific proteins in gall visible as dark bands in the gel. The degeneration of proteins during older stages shows the exit of insect and death of gall tissue.

Keywords: *Alstonia scholaris* R. Br.; Leaf galls; SDS-PAGE; *Pauropsylla tuberculata* Crawf

Introduction

Alstonia scholaris is an elegant evergreen tree of family Apocynaceae, commonly known as devil tree or saptparna grows to height of 30-40 m found in most of parts of India. The plant is used in Ayurvedic, Unani and Sidhha/Tamil types of alternative medicinal systems [1]. Leaves and bark are rich in Echitamine, Echitamine chloride, Scholarine, Scholaricine, monoterpenoid indole alkaloids, iridoids, coumarins, flavonoids, simple phenolics, steroids, saponins and tannins were also found in the plant [2]. The plant is traditionally being used in fever [3], cancer, tumour, jaundice, hepatitis, malaria and skin diseases [4]. One of the important alkaloid present in the plant called alstonine [5] was reported to have anticancerous property [6,7].

Leaves are slightly rounded, leathery, dark green, shortly stalked, lanceolate or obovate, oblong upto 20 cms long and are in whorls of 5-7. Three types of galls have been reported, one each on leaf, fruit and flower of *A. scholaris* [8]. The leaf gall of *A. scholaris* induced by *Pauropsylla tuberculata* crawf., which is an insect belongs to class Psyllidae of order homoptera. Within the Psyllidae, there are nearly 350 widely distributed gall inducing species occurring mainly on the leaves of dicotyledonous plants [9]. Among the insects various hemipteran are known to be capable of inducing galls [10-13].

The development and structure of galls induced by homoptera are generally correlated with the feeding habit of the insects and are predominantly leaf galls. The insects are known to extract nutrients from the phloem, xylem or non-conducting plant cell [10]. Growth of gall tissues are associated with the changes in the levels of their cellular contents such as carbohydrates, proteins, nucleic acids, phenols, IAA and enzymes [14]. The insect activates a perturbation in growth mechanisms and alters the differentiation processes in the host plant, modifying the plant architecture to its advantage [15]. The gall caused by the insect occurs on both sides of the leaf blade of the plant [16], which are covering growth pouch galls. They are semiglobose, conical on adaxial surface of the leaf and truncated conical on the abaxial side. The galls are pale green in the young stages but become yellowish when mature. The gall opens at abaxial side through an ostiole, which is very narrow in immature galls but widens out as the development of the insect proceeds and opens to get the mature insect free (Figure 1a and 1b).

The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique is a powerful tool for estimating the molecular weights of proteins [17]. A major advantage of electrophoresis over morphological evaluation is the speed with which a large number of test samples can be analyzed [18]. It simultaneously exploits differences in molecular size to resolve proteins differing by as little as 1% in their electrophoretic mobility through the gel matrix [19]. Usefulness of the technique depends on the variations within and between test samples. Electrophoretic banding pattern polypeptides can be an efficient approach for assessment of different samples. The present study therefore, explains the existing polymorphism of total proteins through SDS-PAGE to facilitate characterization of different gall stages of *A. scholaris*.

Material and Methods

Periodic collection of the plant material is done for three months after a period of every 15 days and leaf protein was extracted in an extraction buffer (50 mM Tris HCl) followed by centrifugation at 10,000



Figure 1: (a) Healthy and (b) Gall bearing leaves of *A. scholaris*.

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rpm at 4°C for 15 min. Total protein content of the gall stages was estimated in supernatant following Lowry’s method [20] using BSA as standard.

To study the SDS-PAGE protein profile, unidimensional SDS-PAGE [21], (10% separating gel and 5% stacking gel) was carried out in a mini vertical system. For this 100 µg of protein was loaded in each sample well along with 10 µl sample buffer containing bromophenol blue as tracking dye. A medium range marker was also incorporated into the gel to determine the molecular weight of the bands.

The gels were run at a constant voltage of 100 V for 3 hrs followed by staining in coomassie brilliant blue [22] over night. Relative mobility (Rm) of the protein bands was determined and Zymograms were constructed. The gel was photographed and stored in 3% acetic acid.

Results and Discussions

Total protein

The total protein content in the crude extract among the studied healthy and gall stages ranged from 1.8 mg/gm (6th stage) to 3.1 mg/gm (3rd stage) fresh weight of healthy and gall tissue of leaf (Table 1).

SDS protein profile

SDS denatured protein gels could resolve a total of 29 bands in 6 samples of different stages of leaf gall with a sample of healthy leaf which were grouped as 5 distinct SDS protein bands. These SDS protein bands belong to different molecular weight ranging from 44 kDa to 97 kDa. The relative mobility of the bands varied from .23 to .53 in studied stages. Low, medium and high mobility bands were observed in all the cases. One polypeptide band exhibiting relative mobility of .53 representing MW 44 kDa was present in all the stages. Healthy, 1st, 3rd and 4th stages exhibited maximum number of bands i.e. all the five bands were visible, followed by 2nd, 5th and 6th stages which showed 4, 4 and 1 band respectively. A low molecular weight polypeptide band of medium to light intensity with Rm 0.53 (MW 44 kDa) was unique to all gall stages and healthy one. 3rd stage can be differentiated by all other stages due to presence of a strong dark protein band of approximately 97 kDa. 2nd, 5th and 6th stage could be distinguished from healthy, 1st, 3rd and 4th stage by the absence of a protein band of 97 kDa. Variability of protein bands was well expressed in the middle of the gel. The bands in the lower side of the gel were mostly common to all stages. Similarly the protein sub-unit of 97 kDa was not resolved in 2nd, 5th and 6th stage, which helped in differentiating these stages from the rest. The presence versus absence type of polymorphism of SDS protein and these varied intensities was revealed through this study (Figure 2).

Electrophoresis of proteins has been successfully used for the characterization of different taxonomic, evolutionary and genetic relationship studies [23-25].

Leaf gall stages (mg/gm fresh weight of leaf tissue)	Amount of Protein
Healthy	2.1
1 st stage	2.4
2 nd stage	2.7
3 rd stage	3.1
4 th stage	2.9
5 th stage	2.2
6 th stage	1.8

Table 1: Total Protein content in leaf tissue of different leaf gall stages of *A. scholaris*.

Gall Stages	+/- of protein bands (Rm values)					Total
	.23	.31	.33	.46	.53	
H	+	+	+	+	+	5
1 st	+	+	+	+	+	5
2 nd		+	+	+	+	4
3 rd	+	+	+	+	+	5
4 th	+	+	+	+	+	5
5 th		+	+	+	+	4
6 th					+	1
M. W. (kDa)	97	76	64	52	44	29

Table 2: SDS-PAGE Protein Profile of Different Stages of Leaf Gall of *A. scholaris*

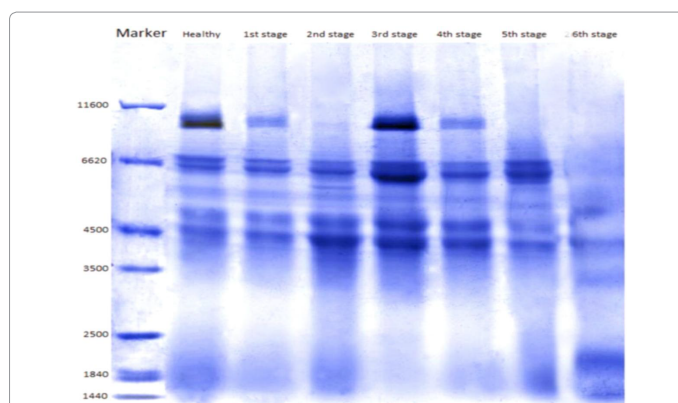


Figure 2: Analysis of total protein content on SDS-PAGE (10 %) showing the variable protein levels at different stages of leaf gall formation in *A. scholaris* (* Showing differences in protein levels at early and older stages).

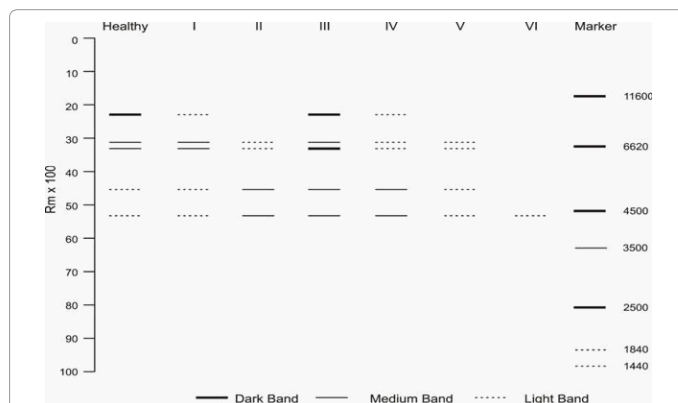


Figure 3: Zymogram of SDS-PAGE protein profile of different stages of leaf gall of *A. scholaris*.

In the present study, the electrophoretic banding profile of total soluble proteins of 6 stages of leaf gall of *A. scholaris* exhibited presence versus absence type of polymorphism, reflecting thereby, and differential synthesis of proteins in the gall at different stages [26] (Table 2). The present investigation on SDS denatured proteins showed differences in number of bands, band width and intensity among different stages of leaf galls of *A. scholaris*. In initial stages of gall the proteins are showing the same banding pattern in the zymogram but in mature stages some extra dark bands are visible, in older stages the number of bands becomes reduced and in last stage only one band is visible [27] (Figure 3).

The present findings confirm the presence of polypeptide bands of heterogenous molecular weight and varying intensity in *A. scholaris* at different leaf gall stages while undergoing the biotic stress. The unburst

galled tissue showed almost two fold increases in the protein content. The protein content showed an initial increase and registered the highest level during the young galled stage of their development and declined thereafter in the mature burst galled tissue where in the insect had already exited out from the chamber [28]. Synthesis of diverse plant proteins are believed to be important in defense mechanism [29].

Conclusion

It can be concluded from the above study that due to interaction between insect and plant tissue certain physiological and biochemical changes occur which lead to hypertrophy and hyperplasia and gall formation takes place. Generation of number of cells requires high amount of protein so the young and mature gall tissue shows high difference in protein concentration as compared to the normal leaf tissue.

This study confirms that when plant is attacked by the pathogens, they inject some elicitors and lead to the synthesis of different type of enzymes and some specific proteins at high amount which is a response of plant against the biotic stress to overcome with it [30]. Insects trigger the defense mechanism of the plant which results the gall formation due to initiation of some biochemical reactions and physiological activities.

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