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Phytoremediative Role of Seaweed Liquid Fertilizeron Protein Profile of *Vigna Unguiculata* under Induced Lead Stress

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Abstract: *Phytoremediative role of seaweed liquid fertilizer (SLF) of Padina pavonia on protein profile of Vigna unguiculata under induced lead stress was studied. SLF and SLF+Pb treatment were found to stimulate the synthesis of new polypeptides (89.13, 79.43, 31.62 and 22.39 KDa) of high and low molecular weight in comparison with control. Stress response proteins (22.39, 31.62, 79.43 and 89.13 KDa) were seemed to be persisting in SLF+Pb treated ones while they were assumed to be disappeared due to long period of exposure to Pb. Also these proteins were not found in control. Thus the results tempted to infer that SLF application retained the stress response protein thereby alleviated Pb stress. However 56KDa protein was prominent in all the samples suggesting that 56KDa protein is insensitive to metal toxicity. 33.8 KDa was found only in control, suggesting that Pb elicited oxidative stress. The study revealed that SLF application mitigated Pb stress by inducing and retaining the synthesis of stress response proteins throughout.*

Keywords: *Phytoremediation, Seaweed liquid fertilizer, Protein profile, Vigna unguiculata, Induced lead stress*

1. INTRODUCTION

Heavy metals are difficult to remove from the environment and unlike many other pollutants they cannot be chemically or biologically degraded and ultimately indestructible. They cause structural [1] and ultra structural [2] alterations in the vegetative organs of plants. However, some metal ions are likely to remain in the cytoplasm and induce oxidative stress via generation of reactive oxygen species (ROS), which will hinder cell metabolism [3]. ROS are known to cause the oxidative modification of proteins and the generation of reactive aldehydes as by-products of lipid peroxidation. Proteins can be affected by ROS either directly by oxidation of amino acid side chains, or by secondary reactions with aldehydicosylation, giving rise to the production of carbonyl-groups in the protein molecule [4]. Modified proteins can undergo a change in their hydrophobicity producing protein aggregates, or giving rise to the formation of polypeptide fragments. Several authors have demonstrated that oxidatively modified proteins are selectively used as target substrates for proteases, and have proposed the used of protein degradation as an index of oxidative stress [5]. Lead (Pb) is a widespread non-essential heavy metal, which enters the ecosystems from natural sources as well as anthropogenic substances. According to the environmental protection agency (EPA), Pb is the most common heavy metal contaminant in the environment [6]. Pradeep Kumar [7] reported that lead causes molecular damage to *Catharanthus roseus* through the formation of reactive oxygen species. Phytoremediation is a sustainable, inexpensive and a viable alternative to conventional remediation methods, and will be most suitable for developing countries like India. In the recent years, seaweeds growing at various localities are explored in different fields due to thepresence of growth promoting hormones, vitamins, amino acids, antibiotics and micronutrients. Rational exploration of seaweeds in phytoremediation can bring out a new eco-friendly technology [8, 9,10,11,12 and 13]. The present work was aimed to study phytoremediative role of seaweed liquid fertilizeron protein profile of *Vigna unguiculata* under induced lead stress.

2. MATERIALS AND METHODS

The brown alga (seaweed), *Padina pavonia* (L.)Lamour*was* collected from Hare Island, Thoothukudi coast, Gulf of Mannar during low-tide period in November2011 – January 2012. It was washed with seawater, followed by fresh water on the spot to remove sand particles and epiphytes. The washed materials were dried in shade at room temperature and powdered. Seaweed liquid fertilizer (SLF) was prepared by adopting the method of Rama Rao [14].

Certified seeds of *Vigna unguiculata* (L.)Walp.obtained from the Agricultural Department, Kovil Patti, Tamil Nadu were sown in plastic pots containing sandy loam soil under natural condition. The following treatments were given as soil administration: control (water), SLF treatment (1% SLF of *P. pavonia*),Pb treatment (Pb 500 ppm), SLF+Pb treatment (1% SLF of *P. pavonia*+Pb 500 ppm). Protein was extracted from leaf samples of forty-day old plants of *V. unguiculata*and were subjected to SDS-PAGE analysis.

2.1. Isolation of Protein

250mg of leaf tissue was homogenized with 0.1M Tris-Hcl buffer (pH 8.0) at 4^oC. The homogenate was centrifuged at 12,000 rpm for 10 minutes using a refrigerated centrifuge (Model REMI-K70) at -14^oC and the supernatant was collected.

2.2. SDS-PAGE Analysis and Determination of Molecular Weights

SDS-PAGE of leaf protein was carried out in vertical slab gel discontinuous buffer system following the method of Laemmli[15] using 10% acrylamide gel concentration. A total volume of 20µl of isolated protein solution was loaded into each well and electrophoresis was carried out at 80V-100V until the bromophenol blue dye reaches the bottom of the gel. After electrophoresis the gel was stained with staining solution comprising 0.2% (w/v) Coomassie Brilliant Blue (CBB) R250 dissolved in 10% (v/v) glacial acetic acid and 90% (v/v) methanol overnight at room temperature. After staining, gel was destained in a solution containing 40% (v/v) methanol, 10% glacial acetic acid and 50% (v/v) double distilled water. Gel was shaken gently until the background of the gel became clear and protein bands were clearly visible. After destaining, the distance travelled by the polypeptides from the well and gel fronts were measured and photograph was taken using a digital camera Nikon COOLPIX L22.The molecular weights of the polypeptides were calculated by using the standard protein marker with molecular weight of 205.0, 97.4, 66.0, 43.0, 29.0, 20.1, 14.3, 6.5, 3.5 and 2.8 Kilo Dalton (KDa).

3. RESULTS AND DISCUSSION

Adaptation of plants to environmental stresses is based on the signal transduction network, from the perception of stress signals to response from gene expression, resulting in metabolic changes [16 and 17]. To substantiate the use of *V. unguiculata* for phytoremediation and for recovering contaminated areas viably it is imperative to analyze the protein profile of *V. unguiculata*. Proteomics is a valuable tool that is becoming increasingly important, complementing the understanding of biochemical and physiological mechanisms in heavy metal stress response. The overall image of the proteome of *V. unguiculata* leaves is shown in Figure (1). The study revealed that SLF and SLF+Pb treatment was found to stimulate the synthesis of new polypeptides (89.13, 79.43, 31.62 and 22.39 KDa) of high and low molecular weight (Table 1). 22.39 KDa protein was seemed to be associated with stress response [18]; persisted for long period in SLF+Pb treated leaves which might have disappeared in Pb treated leaves after long exposure (40 days). The probability of disappearance of this protein is in concordance with El-Khatib*et al*., [19]. He reported that increasing of Cd^{2+}concentration influenced the density of protein bands while long exposure time induced the disappearance of these bands. However, this protein was not found in control plants which confirming22.39 KDa was stress response protein. Other proteins like 31.62, 79.43 and 89.13 KDa were also seemed to be stress proteins persisting in SLF+Pb treated ones while they were assumed to be disappeared due to long period of exposure to Pb. It has been reported that Pb stress could induce changes in tertiary structure of proteins and also increased degradation [20]. Also these proteins were not found in control. Thus the results tempted to infer that SLF application retained the stress response protein thereby alleviated Pb stress. However 56KDa protein, the large subunit of Rubisco was prominent in all the samples which were in concurrent with Gomes *et al*., [18]. He reported the appearance of 56 KDa under Cd^{2+} stress. 33.8

KDa, O_2 evolving protein of photosystem II was found only in the control, suggesting that Pb elicited oxidative stress. The study revealed that SLF application mitigated Pb stress by inducing the synthesis and retention of the stress response proteins throughout.

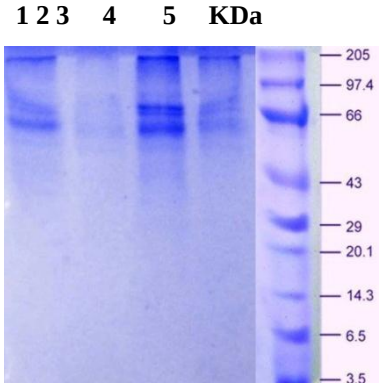

Fig1. *SDS-PAGE analysis of leaf protein profile of V.unguiculata*

Lane 1 = control (Plants grown only with water),

Lane 2 = Pb treatment (Pb 500 ppm),

Lane 3 = SLF treatment (SLF 1% of *Padina pavonia*),

Lane 4 = SLF 1% of *Padina pavonia*+ Pb (500 ppm) treatment,

Lane 5 = Standard Protein Marker, All the treatments were given as soil application.

Table1. *Analysis of protein profile by SDS-PAGE*

Band No.	Molecular weight (KDa)	Rf value	Treatments			
			Control	Pb	SLF	SLF +Pb
1.	112.20	0.182	+	-	+	+
2.	89.13	0.234	-	-	+	+
3.	79.43	0.247	-	-	+	+
4.	70.79	0.273	+	-	+	+
5.	67.61	0.286	+	+	+	+
6.	60.26	0.312	+	-	+	+
7.	56.23	0.338	+	+	+	+
8.	43.65	0.364	+	-	-	-
9.	41.69	0.377	-	+	+	+
10.	33.88	0.416	+	-	-	-
11.	31.62	0.455	-	-	+	+
12.	26.92	0.468	+	-	-	-
13.	22.39	0.519	-	-	+	+
Total	13	13	8	3	10	10

'+' sign indicates the presence of protein band. '–' sign indicates the absence of protein band.

Leaf protein of *V. unguiculata* grown under soil application of seaweed liquid fertilizer (1%) of *P. pavonia*, Pb (500 ppm) and SLF (1%) of *P. pavonia* + Pb (500 ppm) were analysed 40 days after sowing. Control = Plants grown without Pb and SLF.

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