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IMPACT OF FOLIAR APPLICATION OF EXTRACT OF *ULVA RETICULATA* ON FRUIT PROTEIN PROFILE OF *CYAMOPSIS TETRAGONOLOBA* (L.) TAUB.

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ABSTRACT

The changes in protein profile of extract of *Ulva reticulata* grown fruit of *Cyamopsis tetragonoloba* was analysed using Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE). Seaweed extract (Seaweed Extract) treatment was found to stimulate the synthesis of polypeptides 125, 122, 120, 35.3, 7.9 and 6.8 KDa of high and low molecular weight which were not found in control. In control, the polypeptides visualized were 107, 92, 88, 77, 75, 54, 48, 35.6 and 35.4 KDa. Though some differences were observed both in treated and control plants, polypeptides of 92, 88 and 75 KDa were expressed both in treated and control plants with variation in intensity. These results indicate that SWE application as foliar spray increased protein level in fruits by stimulating the synthesis of more polypeptides.

KEYWORDS: Seaweed Extract (SWE), polypeptide, *Ulva reticulata* and SDS-PAGE.

INTRODUCTION

Cluster bean (*Cyamopsis tetragonoloba* L. Taub) is one of the important vegetable crops. The green tender pods are consumed as vegetable. Green pods of cluster bean are rich source of protein, minerals and vitamins. As a rich source of protein and fiber, cluster bean offer several health benefits in vegetable form.[1] To meet the demand of qualitative higher production of vegetables some chemical fertilizers are repeatedly used. Repeated use of chemical fertilizers leads to the hazardous effects on human health and soil health. Therefore, make use of biological fertilizers especially in cluster bean as it is a leguminous crop will beyond doubt give remarkable results. Bio-fertilizers have the ability to mobilize the

nutritionally important elements from non-usable to usable form through biological processes and known to increase yield in several vegetables.[2][3] Seaweed fertilizer is a natural water soluble bioactive material derived from marine macro algae. Seaweed extract (SWE) have been used for several decades to enhance plant growth and productivity by the development of non- pollution organic biostimulants which increase plant growth, vigor, crop yield and quality through increasing efficiency of nutrients uptake. Electrophoresis of protein is a powerful tool for identification of genetic diversity and the SDS-PAGE is particularly considered as a reliable technology because seed storage proteins are highly independent of environmental fluctuations.[4] The present study is aimed to find out the effect of SWE application on fruit protein profile (FPP) which would be a reliable baseline data for distinguishing the upward impact of SWE more precisely.

MATERIALS AND METHODS

Collection of seaweed

Green alga (*U. reticulata* Forsskal) was collected during low tide, at Hare Island, Thoothukudi from November 2014 to February 2015. The sample was washed thoroughly with seawater followed by fresh water to remove sand particles and macroscopic epiphytes. After draining, the seaweed was shade-dried, powdered, sieved and used for the preparation of seaweed concentrate.

Preparation of seaweed liquid fertilizer for foliar application

Seaweed extracts (SWEs) were prepared by adopting the method of[5] with certain modifications. About 20g dried seaweed powder with 200ml distilled water was heated to 60^{o}C and maintained at the temperature for 24 hr in a hot air oven. The extract was filtered and then centrifuged at 10000 rpm to remove suspended impurities. The filtrate was stored in air tight bottles at 4^{o}C (100% seaweed concentrate) for further use.

Experimental design

A pot culture experiment was conducted during February to April 2015 at Plant Research Centre, St. Mary's College Campus, Thoothukudi, Tamil Nadu, India. The pots were filled with 3kg of garden soil. 20 seeds were sown in each pot. After the emergence of seedlings, they were thinned to ten plants per pot and allowed to grow upto fruiting stage. Weeding and watering were done at regular intervals. 1% SWE was applied as foliar spray (along with 100ml of distilled water in the ratio of 1: 100) after expansion of first leaf and was continued till fruiting stage. Enough replicates were maintained.

Isolation of protein

1g fruit tissue was homogenized with 0.1M Tris-HCl buffer (pH 8.0) at 4^{o}C. The homogenate was centrifuged at 12,000 rpm for 10 minutes using a cooling centrifuge (Model REMI-K70) at -14^{o}C and the supernatant was used as protein sample.

SDS-PAGE Analysis and Determination of Molecular Weight

SDS-PAGE was carried out in vertical slab gel discontinuous buffer system following the method of[6] 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 the dye reached the bottom the gel was removed and was stained with staining solution comprising 0.2% (w/v) Coomassie Brilliant Blue (CBB) R-250 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 each polypeptide from the well and gel fronts were measured. Gel was placed on a white light transilluminator and photographed using a digital camera SONY Cyber-Shot 10.1.

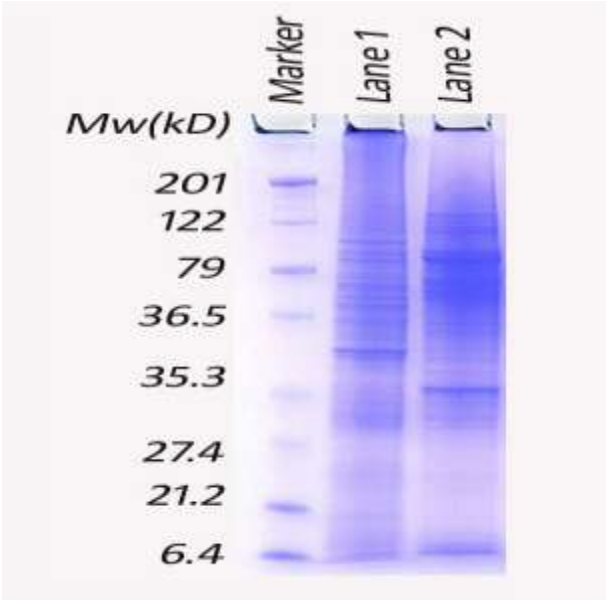

Plate 1. SDS-PAGE analysis of fruit protein profile of *C. tetragonoloba.*

Lane1- Plants grown with water.

Lane2- Plants grown with extract of *U. reticulata* (1%) as foliar spray.

Table. 1 Fruit protein profile of *C. tetragonoloba.*

Band No.	Molecular Weight (KDa)	Rf value (cm)	Treatments	
			Control	Treated
1	125	0.13	-	+
2	122	0.15	-	+
3	120	0.16	-	+
4	107	0.18	+	-
5	92	0.21	+	+
6	88	0.26	+	+
7	77	0.31	+	-
8	75	0.34	+	+
9	54	0.35	+	-
10	48	0.37	+	-
11	36.1	0.39	-	+
12	35.8	0.41	-	+
13	35.6	0.45	+	-
14	35.4	0.46	+	-
15	35.3	0.47	-	+
16	7.9	0.55	-	+
17	6.8	0.68	-	+
Total	17	17	9	11

'+' sign indicates the presence of protein band '-'sign indicates the absence. Fruit protein of *C. tetragonoloba* grown under foliar application of *U. reticulata* extract (1%) was analysed by SDS-PAGE. Control=Plants grown without SWE application.

RESULTS AND DISCUSSION

Seaweed has long been established that seaweed liquid fertilizer (SLF) in different forms as 'organics' increased growth and yield potential of agricultural crop species.[7][8][9][10] Widespread review's did not show much attention on quality of various plant's parts that are consumed daily. In this present investigation fruit protein was analysed by using SDS-PAGE, a reliable technique, because (i) fruit storage proteins are highly independent of environmental fluctuations (ii) fruit storage protein variability explicit the long term impact of seaweed extract (SWE). The high stability of fruit storage protein profile makes it a promising tool for distinguishing the efficacy of SWE from other environmental variables. Proteomics is a valuable tool that is becoming increasingly important, complementing the understanding of biochemical and physiological mechanisms behind SWE as biostimulant and separation performed using one dimensional gel electrophoresis visualized the high and low molecular weight/polypeptides (Plate 1). Extract of *U. reticulata* treatment was found to stimulate the synthesis of polypeptides (125, 122, 120, 36.1, 35.8, 35.3, 7.9 and 6.8 KDa) of high and low molecular weight (Table 1). In control, the polypeptides visualized were 107,

92, 88, 77, 75, 54, 48, 35.6 and 35.4 KDa. Though some differences were observed in both treated and control with variations in intensity. 125 KDa protein has affinity for sterol regulatory sequence.[11] 120 KDa glycol protein functions such as cell growth and development, maintaining structural integrity of cell wall defense, cell-cell communication.[12] These polypeptides were expressed in SWE treated fruit which were not found in control. Fruit storage protein variability explicated the long term impact of SWE. The high stability of fruit storage protein profile makes it a promising tool for distinguishing the efficacy of SWE from other environment variables. The results of the present study clearly indicate that SWE of *U. reticulata* stimulated to synthesis more polypeptides on fruit protein profiling of *C. tetragonoloba*.

CONCLUSION

From the above results, it is concluded that foliar application of extract of *U. reticulata* in *C.tetragonoloba* was found to be more suitable for inducing more polypeptides.

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REFERENCES

1. Deshmukh RP, Nagre PK, Wagh AP, Dod VN. Effect of different Biofertilizers on Growth, Yield and Quality of Cluster bean. *In J of Adv in Plant Res.*, 2014; 1(2): 39-42.
2. Kumar R, Gupta PP, Jalali BL. Impact of VA-mycorrhiza, *azotobacter* and *rhizobium* on growth and nutrition of cowpea. *J of Mycolo and Pla Patholo*, 2001; 31(1): 38-41.
3. Khandelwal R, Choudhary, SK., Khangarot, SS, Jat, MK, Singh, P. Effect of inorganic and bio-fertilizers on productivity and nutrients uptake in cow pea [*Vigna unguiculata (L.) Walp*]. *Legu res*, 2012; 35(3): 235 -238.
4. Javaid A, Ghafoor A, Anwar A. Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. Pak J Bot, 2004; 36: 87-96.
5. Rama Rao K. Preparation, properties and use of liquid seaweed fertilizer from *Sargassum*. In: Workshop on Algal Products and seminar on Phaeophyceaein India, Seaweed Research and Utilization Association, Madras, India: 1990; pp. 7-8.
6. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Natur*, 1970; 227: 680-685.

7. Erulan V, Sourndarapandiyan P, Thirumaran G, Ananthan G. Studies on the effect of *Sargassum polycystum* extract on the growth and biochemical composition of *Cajanus cajan* (L.) Mill sp, Am Eur J Agri Environ Sci, 2009; 6: 392-399.
8. Usha R, Rani SMV. Phytoremediative role of seaweed liquid fertilization protein profile of *Vigna unguculata* under induced lead stress. Int J of Res Stu in Biosci, 2014; 2(11): 164-168.
9. Kumareswari K, Rani SMV. Utilization of seaweeds to enhance growth and nutritive status of *Amaranthus caudatus* L. Int J of Res Stu in Biosci, 2015; 3(3): 9-15.
10. Kumareswari K, Rani SMV. Nutritional superiority of seaweed based organic green leafy vegetable, *Amaranthus retroflexus* L. Int J of Pharma and Biosci, 2016; 7(1): (B) 332-335.
11. Kaul D, Kaur M. Receptor-Ck regulates membrane-bound 125 KDa protein having affinity for genomic sterol regulatory sequence. *Mol. Cell Biochem*, 2001; 216(1-2): 141-3.
12. Wu, H., B. deGraaf, C. Mariani and A.Y. Cheung. Hydroxyproline-rich glycoproteins in plant reproductive tissues: structure, functions and regulation. *Cell Mol Li Sci*, 2001; 58: 1418-1429.