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STUDIES ON AQUEOUS EXTRACTS OF THREE GREEN ALGAE AS AN ELICITOR OF PLANT DEFENCE MECHANISM

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Abstract

Codium elongatum, Caulerpa texiflora and *Ulva lactulus* (green algae) were collected from Karachi coast. High Molecular Weight Crude Elicitor Preparation (HMWCEP) of these plants were obtained by sequential extraction of H_2O, dilute NaOH and HCl, ethanol precipitation and freeze drying. Results showed that yields were high in NaOH extracts ranging from 4.3 - 69.6%. These HMWCEP were chemically analysed for total sugar, protein, SO_4 group and uronic acid content. Elicitor activity was determined in terms of induced browning in *Cicer arietinum* (chickpea) tissues. Amongst the three algae HMWCEP of *Codium elongatum* was found the most active elicitor and produced maximum browning after 24 hour incubation at 25°C.

Introduction

The biochemical basis of defence mechanism confering disease resistance in plants is now under active investigation (Dixon & Christopher, 1990). Natural or synthetic molecules that are able to elicit, induced resistance in plants, are grouped together and known as "Elicitors" (Keen *et al.*, 1972). This phenomenon opens the possibilities of preventing diseases by stimulating the plants natural defence mechanism and thus limiting pathogen spread (Darvill & Albersheim, 1984; Iqbal & Paxton, 1991). Literature survey revealed that in most cases elicitor activity was associated with the polysaccharide fractions of various preparations obtained from fungal cell wall and culture filtrate (Hamdan & Dixon, 1987). Keeping in view that seaweeds generally comprise of 40-69% of carbohydrate we found worth exploring these polysaccharides as such or after hydrolysis (the degraded products) as an inducer of hypersensitive response (Arnold & Merlin, 1990) characteristics to resistance mechanism of plant against diseases. The occurrence of various seaweeds at the Karachi coast has been reported by Usmani *et al.*, (1986).

The purpose of the present investigation was to follow the extraction process of seaweeds, quantitatively with the analysis of various extracts for total contents of sugar, protein, sulphate and uronic acid, to provide a description of compositional differences more labile to different extracting media. High Molecular Weight Crude Elicitor Preparations (HMWCEP) were obtained by ethanol precipitation of polysaccharides and freeze drying. These HMWCEP were evaluated for their elicitor activity.

Material and Methods

Collection and pretreatment of Algae: Green algae viz., *Codium elongatum, Caulerpa texiflora* and *Ulva lactulus* were collected from Buleji and Cap Monz in the month of November, 1993 and January, 1994. The samples of fresh seaweed were washed with running tap water, air dried under shade on netted wooden trays.

Water, Moisture and Ash Content of Seaweeds: Dry yield and water content of each alga was determined by thorough washing and drying at room temperature under shade, till constant weight. Moisture content was determined by heating 1 gm of air dried sample at 110°C in the oven, till constant weight. For ash 0.5 gm of each sample was heated on burner to remove soot and then burnt in furnace at about 600-800°C for 2 hours, another 2 hrs heating was carried out for complete ashing.

Sequential Extraction of Polysaccharides from Green Algae: One gm of dried material was chopped and dipped into 100 mL distilled water, magnetically stirred for 16-18 hr at room temperature, filtered through No.1 Whatman filter paper and stored frozen till further processing. The residue was re-extracted with 100 mL water at 60-65°C for another 12-16 hr, filtered and stored at -20°C (Hot extract). Another 1 gm of the substance was subjected to cold/hot extraction under similar conditions as described above using 100 mL of 0.1N NaOH and 0.1N HCl and the extracts were stored frozen.

Crude Elicitor Preparations from Seaweed Extracts: Acid, alkali and aqueous (cold/hot) extracts of each algal variety were treated with 1.3 volume of distilled ethanol and samples were stored at 4-6°C for 3-4 days to precipitate out polysaccharides. The supernatant was discarded and the precipitate (HMWCEP) collected by centrifugation at 3000-4000 rpm for 20-30 minutes and dissolved in minimum quantity of water. Dry weight of HMWCEP were obtained by lyophilization using a bench top model of Lab. Conc. Freeze drier equipped with an Edward high vacuum pump.

Chemical Analysis of HMWCEP of Seaweed: Total carbohydrate content of these HMWCEP was determined by the phenol sulphuric acid method (Dubois *et al.*, 1956). Total protein was determined by Bradford method (1976), bovine serum albumin was used as standard. Assay for sulphate group was done by the method of Dodgson (1961). Total acidic sugar was determined by uronic acid carbazole method (Bitter & Muir, 1962).

Acid Hydrolysis and Monosaccharide Composition of HMWCEP: 100 μL of stock (1 mg/ml) solution of HMWCEP of each algal genus under study was taken into air tight sealed tubes and dried over KOH pellets in a desicator maintained under vacuum. 100 uL of 90% formic acid was added, flushed with nitrogen and heated at 100°C for 8 hr. After hydrolysis samples were left overnight in vacuum desicator over P_2O_5. Dried hydrolysate were resuspended into 50 uL of distilled water. Paper chromatography was carried out on Whatman No. 1 paper with Butanol: Acetic acid: Water (4: 1: 5), solvent system. Chromatograms were developed with silver nitrate reagent (Georg & William, 1974).

Germination of Seeds and Elicitor Treatment: A general method of elicitor application was employed in all experiments, as previously described by Whithead *et al.*, (1982). Chickpea seeds were germinated in a tray on filter paper placed on a moist cotton bed

Table 1. Water moisture and ash contents of *Codium elongatum* *Caulerpa texiflora* and *Ulva lactulus*.

Name of Algae	Aron of Collection	Time of Collection	Expressed as g% H_2O Cont.	Moisture	Dry Ash
Codium elongatum	Buleji	Nov.1993	95.1	6.44	10.0
Caulerpa texiflora	Cap Mohz	Jan. 1994	88.21	8.1	20.0
Ulva lactulus	Same as above	Same as above	95.4	7.68	14.0

at 25°C in the dark. The excised cotyledons (2-3 days) were surface sterilized by immersion in 1% sodium hypochlorite for 2 minutes and then washed extensively with distilled water, finally rinsed with sterile water, elicitor preparation at a concentration of 100 ug glc eq/mL were used. Treated and control samples were prepared by application of 20 uL of test solution and sterile H_2O (control) to the cut surface of cotyledons placed in a Petri dish (10-15 cotyledons) on a moistened filter paper incubated at 25°C in the dark for a specified time period of 24 hours.

Results and Discussion

Miosture content of green alga, *Codium elongatum*, *Caulerpa texiflora* and *Ulva lactulus* were found in the similar range (6.4-8.1%) as reported by Qari (1988), whereas water content of *C. elongatum* and *U. lactulus* were high (95.1 and 95.4% respectively). This may be due to the tubulur structure of *C. elongatum* and fluffy, leafy structure of *U. lactulus*. Ash contents were in the range of 10-20% (Table 1).

Separation of polysaccharides in algae is often possible by sequential extraction and is useful to provide information on the range of polysaccharide present (Percival & McDowell, 1990). Generally yields of HMWCEP were high (13.4-69.6%) in the cold/hot fractions of NaOH extracts (Table 2) as earlier reported by Mian & Percival (1973), with some exceptions that very low yields were observed in hot alkali fraction of *C. elongatum* and cold alkali fraction of *C. texiflora*, ranging from 9.3 and 4.3%, respectively. The difference was observed in the yield of cold/hot acidic extracts of *C. elongatum* 2.9-28.0%. Considering these results it is concluded that under specified conditions NaOH is the best extracting medium.

Chemical analysis of HMWCEP of these algal genera showed that higher sugar content 18-66% were found in all three extracts (cold/hot) of *U. lactulus* in comparison to other two algae (Table 2). It was also observed that carbohydrate content was high in cold aqueous extract of each genus ranging between 28-64%. Protein was found in most fractions of *U. lactulus*. A low protein content (0.5-1.6%) appeared in some frac-

Table 2. Dry yield and Chemical Composition of HMVCEP of *Codium elongatum*, *Caulerpa texiflora* and *Ulva lactulus* (Expressed as g%).

Name of algae	Extracts	Dry yield C/H	Sugar C/H	Protein C/H	Uronic acid C/H	SO4 C/H
Codium elongatum	H_2O	3.8/2.1	32/28	NF/0.5	1.4/0.9	4.0/8.0
	0.1N NaOH	30.0/9.3	12/14	NF/1.0	0.5/0.7	17/2.0
	0.1N HCl	2.9/28.0	30/76	NF/NF	1.0/2.0	35/19.0
Caulerpa texiflora	H_2O	5.67/3.54	28/20	NF/1.0	1.2/4.2	46/20
	0.1N NaOH	4.3/13.42	14/10	1.6/NF	1.2/0.4	9.0/39
	0.1N HCl	3.0/4.6	55/22	0.5/1.5	4.2/10	40/23
Ulva lactulus	H_2O	14.8/3.86	64/44	2.0/1.0	8.0/5.0	20/3.0
	0.1N NaOH	55.5/69.6	33/18	1.4/2.1	2.2/1.0	38/26
	0.1N HCl	-/3.34	-/66	-/1.5	-/3.2	-/5.0

NF = Not found, C/H = Cold/Hot

tions of *C. elongatum* and *C. texiflora*. Various polysaccharides of many green algae are complex in nature. Water soluble and other heteropolysaccharide contain a wide variety of sugars and in some genera glucuronic acid (Percival & McDowell, 1967; Percival, 1978) and uronic acid was found in various proportion in all the cold/hot fractions of different extracts of green algae. Investigated values were high (4.2-10.0%) in cold/hot acid extracts of *C. texiflora* and cold/hot aqueous extracts of *U. lactulus*, 8-5.0% respectively. Similarly SO_4 was present in each extract of these three algal genera. Higher SO_4 values could be seen in *C. texiflora* (9-46%). Surprisingly very low SO_4 was found in NaOH hot fraction of *C. elongatum* and hot aqueous fraction of *U. lactulus* such as 2-3% respectively.

Water soluble and other heteropolysaccharide of many green algae are complex in nature (Percival & McDowell, 1967). Acid hydrolysis and paper chromatography of these species showed that glucose, rhamnose, arabinose, xylose and fucose were found in varying proportions in various extracts. Glucuronic and galactoronic acids were present in some cold/hot fractions of *C. elongatum*, with negligible amount of these acids present in hot acid extracts of other two genera. Similarly galactose and mannose were found in only acidic fractions of *U. lactulus* and *C. elongatum*.

The extent of induced browning is a qualitative measurement of elicitor activity. HMWCE preparations of various extracts of green algae under study were tested for their elicitor activity. It was observed that *C. elongatum* showed prominent browning as compared to *C. texiflora* and *U. lactulus*. Intense browning was produced in its hot aqueous cold alkali and cold acidic extracts. On the other hand only hot NaOH fraction of *C. texiflora* and hot aqueous fractions of *U. lactulus* showed sufficient browning,

Table 3. Elicitor activity in terms of induced browning by *Cadium elongatum, Caulerpa texiflora* and *Ulva lactulus*.

Chlorophyceae (Green algae)	Cotyledons treated with	Elicitor Activity	
	Extracts	Cold	Hot
Cadium elongatum	H_2O	++	+++
	0.1N NaOH	+++	++
	0.1N HCl	+++	++
	Control Wound	+/+	
	Control H_2O	O/+	
Caulerpa texiflora	H_2O	++	++
	0.1N NaOH	++	+++
	0.1N HCl	++	++
	Control Wound	+/++	
	Control H_2O	+/++	
Ulva lactulus	H_2O	++	+++
	0.1N NaOH	++	++
	0.1N HCl	-	++
	Control Wound	0/+	
	Control H_2O	+/++	

Data are mean of two replicates and findings of two determinations.
Degree of Browning = 0/+ for least browning.

while remaining fraction of these two algae showed less browning equal to that of controls (Table 3).

Treated tissues of *C. arietinum* responded differentially to various preparations of three algal genera. The extent of browning produced was different for various samples. This could be due to compositional differences of these preparations. It is possible that fractions may have some structure, which are more likely to interact with the receptor molecules of *Cicer arietinum*.

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