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ESTIMATION OF INDUCED SECONDARY METABOLITES IN CHICKPEA TISSUES IN RESPONSE TO ELICITOR PREPARATION OF SEAWEEDS

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Disease response of plants in terms of induced browning and phytoalexin (induced secondary metabolites) production were recorded in the tissues of *Cicer arietinum* (Chick pea) treated with the High Molecular Weight Crude Elicitor Preparations, HMWCEP "Polysaccharides" of *Hypnea musciformis* (red algae), *Padina tetrastromatica* (brown algae) and *Ulva lactulus* (green algae). A UV-visible spectrophotometric method has been developed for the quantification of induced secondary metabolites with time.

Key words: Induced secondary metabolites, Phytoalexin, Elicitor activity, *Cicer arietinum,* UV Scan.

Introduction

In response to any infection or stress, plants exhibit some natural resistance mechanism (Darvill and Albersheim 1984, Joseph 1995). Disease resistance involves not only static protection but also some inducible defense or hypersensitive responses leading to tissue browning with cell necrosis, synthesis and accumulation of aromatic phenolic compounds and phytoalexin production, induction of phytoalexin biosynthetic enzymes, leakage of electrolytes from isolated cells, ethylene production and accumulation of hydroxy proline rich glycoprotein. Amongst the induced browning, synthesis and accumulation of phytoalexin 'low molecular weight antimicrobial compounds' have especially gained attention. Phytoalexins have been isolated and characterized from a wide variety of plants (Iqbal and Paxton 1991) and their role in disease resistance has been deduced from their antifungal activity (Arnold and Merlin 1990). Accumulation of medicarpin and macckian (yeast extract, *A. rabie* polysaccharides) is an induced process involving *de novo* enzymatic synthesis for accumulation of increased level of phytoalexin on demand (Gunia *et al* 1991).

Induction of resistance response has been achieved with molecules that have been isolated from the cell wall culture filtrate and cytoplasm of various parasitic plant pathogens. These molecules are called elicitors (Keen *et al* 1972) and include also host derived endogenous elicitors (Legendre *et al* 1993) and abiotic elicitors (Baily and Berthier 1981). Substances of microbial origin are diverse in nature and include polysaccharides (Gunia *et al* 1991), oligosaccharides (Clarence and Edward 1991), proteins (Vogelsang *et al* 1994) and fatty acids (Castoria *et al* 1995).

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In the present study chick pea *(Cicer arietinum)* tissues were treated with the High Molecular Weight Crude Elicitor Preparations HMWCEP (polysaccharides) obtained from various algal plants *viz: Hypnea musciformis, Padina tetrastromatica* and *Ulva lactulus.* The elicitor activity was determined in terms of induced browning and production of induced secondary metabolites estimated by UV-visible spectrophotometric method.

Materials and Methods

Plant collection, extraction and dry weight determination of High Molecular Weight Crude Elicitor Preparations (HMWCEP) from various algal plants were carried out as described earlier (Fatima and Seema 1999). 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 carried out by the method of Dodgson (1961). Total acidic sugar was determined by uronic acid carbazole method (Bitter and Muir 1962).

Elicitor treatment. A general method of elicitor application was used as earlier described (Whitehead *et al* 1982). Chick pea seeds were soaked overnight in distilled water and germinated on filter paper placed on wet cotton, incubated at 25°C in dark. Cotyledons of 3 day old seedlings were surface sterlised by immersion in 1% sodium hypochlorite for 2,3 minutes and then washed extensively with sterile water. Initially elicitor preparations at a concentration of 100 µg glu eq ml^{-1} were used. Treated and control samples were prepared by application of 20 µl of test solution and sterile water to the cut surfaces of cotyledons, placed in petri dish (10-15 cotyle-

dons plate^{-1}) on a moist filter paper incubated at 25°C, kept in the dark. The browning was recorded after 24h of incubation. In another experiment each of the treated and control cotyledons were incubated for 6, 15, 24, 48, and 72 h in dark. The given elicitor activity data is average of duplicate samples and findings of two determinations (Table-1).

Extraction and estimation of induced secondary metabolites. After specified time of incubation the cotyledons were dipped into 15-20 ml, redistilled ethanol (95%) and left overnight for complete extraction. Illumination was avoided as much as possible. The extract was filtered through Whatman filter paper no. 1 in a 25 ml volumetric flask and diluted with 95% ethanol upto the mark. 50 µl of this stock solution further diluted by adding 3 ml of absolute alcohol, and a blank was prepared by taking 3 ml of this diluted solution.

Instrument. UV-spectra were recorded on Lambda 4C UV/ Visible spectrophotometer (Perkin Elmer) of variable wave length. The results were obtained in terms of absorption intensity of various alcoholic extracts scanned at a wavelength of 190-550 nm. Absorption intensity of peak A at 254 nm (Fig 1), assumed to be proportional to the amount of induced secondary metabolites, has been calculated per gram fresh weight of cotyledons.

Results and Discussion

Dry chopped plants were extracted and HMWCEPs were obtained by ethanol precipitation and lyophilization. Yield was high (55.5%) in cold NaOH extract of *U. lactulus* (green algae). Chemical analysis of HMWCEP preparations showed that total sugar and protein contents were high in *U. lactulus* as compared to *H. musciformis* (red algae) and *P. tetrastromatica* (brown algae), (Table 1). Increased amount of uronic acid was found in *P. tetrstromatica.* Initially elicitor activity of these preparations was recorded in terms of induced browning in chickpea tissues after 24 h of incubation and the degree

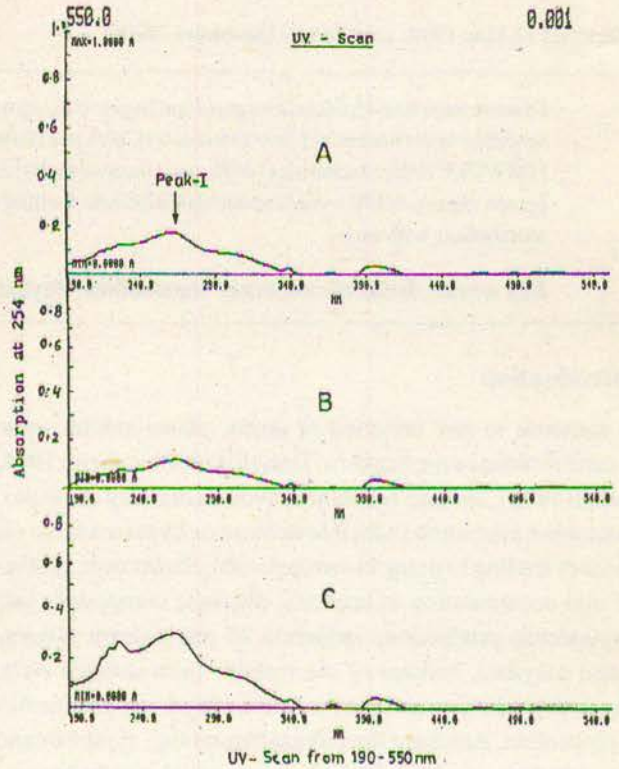

Fig 1. UV scanning of ethanolic extracts of elicited chickpea tissues for estimation of induced secondary metabolites. A, Control wound; B, Control water; C,*U. lactulus* (NaOH cold extract).

Table 1
Yield and chemical composition (expressed as g%) and elicitor activity of HMWCEP obtained from different algal plants

Name of algae	Dry wt of HMWCEP	Total sugar	Total protein	Uronic acid	Total SO_4	Elicitor activity S/D
H. musciformis 0.1N HCl cold ext.	20.1	8	1.2	1.6	7.9	++++/+++
P. tetrastromatica 0.1N NaOH cold ext.	25.5	4	N.F	6.6	28	+++/+++
U. lactulus 0.1N NaOH cold ext.	55.5	33	1.4	2.2	38	++/+++
Cont. Wound	--	--	--	--	--	++/+
Cont. H_2O	--	--	--	--	--	+/0

Degree of Browning = 0/+ indicate least browning for samples/duplicate, NF, Not Found; S/D, Sample/duplicate.

Table 2

UV absorption of induced secondary metabolites of chickpea tissues treated with HMWCE preparation of various seaweeds

Absorption of alcoholic extracts at λ 254/g fresh wt. of treated and control tissues of chickpea

Incubation time (h)	Control wound	Control sterile H_2O	#*H musciformis*	*P. tetrastromatica*	$*U. lactulus$
6	0.163	0.170	0.248	0.330	0.269
15	0.307	0.239	0.381	0.348	0.379
24	0.352	0.089	0.519	0.636	0.440
48	0.530	0.270	1.023	0.768	0.424
72	0.909	0.442	1.064	0.844	0.553

Results obtained by averaging duplicate samples. # Red algae (cold acid extract), * Brown algae (cold alkali extract), $ Green algae (cold alkali extract).

of browning exhibited was in the order of *H. musciformis* > *P. tetrastromatica* > *U. lactulus* (Table 1).

It is clear from numerous papers (Kessman *et al* 1988; Joseph 1995) which provide evidence quite convincingly in support that the rate at which a phytoalexin accumulates in a particular host pathogen combination is probably more important than the final concentration and is a characteristic of resistance response. The high extinction coefficient of many phytoalexins in the ultraviolet region of spectra allows quantification of induced secondary metabolites (Osswald 1985). In this study the alcoholic extracts of treated and control tissues of *Cicer arietinum* were analysed for changes in concentration of induced secondary metabolites with time in response to the treatment of various algal preparations under investigation. Results in Table 2 indicate that concentration of induced metabolites were measured as early as 6 h of incubation; elevated values were observed in the treated samples as compared to the controls. A gradual increase in the production of induced secondary metabolites was observed in the samples treated with the elicitor preparation of *H. musciformis* (red algae) throughout the incubation period. No significant changes (0.018 and 0.016) were found between 6-15 and 24-48 h of incubation period in the samples treated with HMWCEP of *P. tetrastromatica* (brown algae) and *U. lactulus* (green algae) respectively. In some cases 2-3 fold increase was observed at later stages i.e. 48-72 h of incubation as compared to the values of control. In our experiments in most cases the values of controls were not zero. Lower values were observed for the controls treated with sterile water as compared to wounding only. This could be due to some added dilution factor of water. In Table 2, absorption value 0.089 for control H_2O at 24 h of incubation is very low in series and found no explanation for this eratic results could be found and it may be related to the physiological condition of the tissues.

These experiments revealed that treated tissues of chickpea responded differently to various preparations of algae and produced a positive and definite elicitor activity. The extent of browning and level of induced secondary metabolite was different for various samples; it could be due to the compositional and structural differences of these polysaccharides. The UV-Spectrophotometric method has been successfully used for the estimation of induced secondary metabolites; especially the amplitude and timing of response have been examined by this method.

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