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Carrageenan as an elicitor of induced secondary metabolites and its effects on various growth characters of chickpea and maize plants

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Abstract The effects of polysaccharide elicitor k-carrageenan obtained from *Hypnea musciformis*, red algae on the production of Induced Secondary Metabolites, ISMs (the disease resistance compounds) and on various growth characters of chickpea and maize plants were studied. Experiments were conducted in the field of PCSIR Laboratories Complex Karachi during December 2008–April 2009 in randomized complete block design with three replications. Three elicitor treatments were used, a solid preparation in which the elicitor was mixed with soil (T_2 1 mg/g) and applied around the seeds in the soil. The two other preparations were liquid, T_1 and T_3 at a concentration of 100 μg glc eq ml^{-1} and were applied around the sowing seeds and as a foliar spray on the plants, respectively. Statistical analysis of the data revealed that these treatments significantly enhanced all the growth characters of chickpea except T_2 that gave the nonsignificant difference in the plant height. Maximum plant height (80.3 cm), number of pods plant^{-1} (76.2), number of branches plant^{-1} (25.0), number of leaves plant^{-1} (125.6), earlier flowering and high ISMs contents in leaves, stem and grains of chickpea were recorded in T_1 treated chickpea plants. In maize plants only T_1 and T_3 treatments (with minor exceptions) had significant effects on few characters like plant height, stem diameter, number of leaves plant^{-1} and on ISMs contents in leaves while number of cobs plant^{-1} and flowering time were nonsignificantly affected by these treatments. These results suggested that k-carrageenan elicitor can be used as a potent plant protectant as well as growth promoting agent especially for chickpea plants.

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1. Introduction

In recent years there has been an upsurge of research activities on the biocontrol of plant diseases. Biological control methods of protecting crops from pests and pathogens using naturally occurring bacteria, fungi and viruses are recently reported (Kapooria, 2007; Jayaraj et al., 2008). These agents protect the crops from infection by pathogens without affecting the growth and ultimate harvesting of the crops (Ning et al., 2003).

The most effective way of achieving crop protection is to introduce the desired resistance into the plants by activating or eliciting their natural defense system which is an environmentally safer approach to the problem. The potent elicitors described in literature are diverse in nature including polysaccharides, oligosaccharides, peptides, proteins and lipids and are derived from cell wall, culture filtrate and cytoplasm of various parasitic and nonparasitic plant pathogens (Rao et al., 1996; Halim et al., 2004). In most cases elicitor activity is associated with polysaccharide fraction of various preparations (kessman et al., 1988). It is documented that polysaccharides purified from seaweeds as well as derived oligosaccharides have the ability to trigger plant defense responses (Kloareg and Quatrano, 1988; Potin et al., 1999).

Carrageenans, the algal polysaccharides are the sulphated galactans consisting of 1,3 and 1,4 linked galactose residues and divided into three main classes kappa, iota and lambda carrageenans. Mercier et al. (2001) reported that λ-carrageenan elicited an array of plant defense responses possibly through the effect of its high sulphate content and found to induce signalling and defense gene expression in tobacco leaves. K-carrageenan also found to elicit ß-1,3 glucanase activity in *Rubus fruticosus* cell suspension cultures (Patier et al., 1995).

Chickpea (*Cicer arietinum* L.) and maize (*Zea mays* L.) are the two oldest and most important food and feed crops in Pakistan. They are a rich source of good quality protein and other nutrients. The present study is a continuation of the research work carried out to enhance the defense mechanism of plants by the application of polysaccharide elicitors obtained from red, brown and green seaweeds (Bi and Iqbal, 2000). In this work the effect of k-carrageenan, the polysaccharide elicitor obtained from *Hypnea musciformis*, red algae was studied in terms of semiquantitative induction of secondary metabolites in chickpea and maize plants grown in the field. Data were also collected on the responses of various growth characters of these plants towards the elicitor treatments.

2. Material and methods

Seeds of chickpea and maize plants were purchased from the local market of Karachi. Crops were grown in randomized complete block design replicated thrice in the experimental field of PCSIR Laboratories Complex Karachi. Experiments were carried out from December 2008 to April 2009. The minimum and maximum temperatures during these experiments were 25–32 °C. Each plot had an area of 15 ft^2. Five plots were used for each plant, three for treated and two for the control plants. Water supply was regulated once a week in the beginning and then after 10 days interval. Six healthy and uniform sized seeds of maize and ten of chickpea per plot were planted at a proper distance from each other in the first week of December. Crops were harvested in April.

Soil analysis showed that it contained sand 73.8%, clay 10.9% and slit 10.7%. Its texture was loamy sand and has organic matter 3.1% and $CaCO_3$ 14.9%. pH of the soil was 7.8 and its moisture content was 6.1%.

2.1. Preparation of elicitor treatments from *H. musciformis*

1. Liquid elicitor preparation at a concentration of 100 µg glc eq ml^{-1}(A) was described previously (Bi and Iqbal, 1999).
2. Solid elicitor preparation (B) was prepared by mixing dry hot aqueous extract with soil (1 mg/g).

2.2. Method of application of elicitor treatments

50 ml of A (Treatment, T_1) and 5 g of B (Treatment T_2) were separately applied around each seed at the time of sowing. Similarly foliar spray of 5 ml of A (T_3) was applied after the first appearance of plants and then once a week during the experiment, volume of spray solution was gradually increased with the plant height. Control plants were treated with distilled water. The data were collected after selecting four normal sized plants from each plot for measuring some parameters like plant height (cm), number of leaves $plant^{-1}$ and days to flowering for both plants; number of pods $plant^{-1}$ and number of branches $plant^{-1}$ for chickpea and number of cobs $plant^{-1}$ and stem diameter (mm) for maize plants separately.

2.3. Extraction and Estimation of Induced Secondary Metabolites (ISMs)

0.5 g dried leaves, stem and grains of treated and control plants of chickpea were ground and extracted with 4 ml of 1 M HCl on boiling water bath for 30 min. Extracts were filtered through whatman filter paper No.1 and defatted twice with n-Hexane (2 ml). The aqueous layers were separated by centrifugation and dried on water bath. The known quantity of the dried extracts were dissolved in 10 ml methanol–water mixture 60:40 and passed through small silica column to obtain clear solution. 150 µL of this solution was further diluted with 10 ml of distilled ethanol (95%) in a volumetric flask for recording the UV absorbance. The same procedure was repeated with 0.5 g leaves of treated and control maize plants. Total ISMs were estimated twice during the experiment (after 60 days each time).

2.4. Instrumentation

UV spectra were recorded on a UV–Visible Spectrophotometer (Specord 200) of variable wave lengths. The results were recorded in terms of absorption intensity of various alcoholic extracts scanned at the wavelength of 190–500 nm using ethanol as the blank.

2.5. Statistical analysis

Difference between the means of each treatment was statistically analyzed with the difference between the means of each treatment and also with the means of control with a quantity called the Least Significant Difference (LSD).

3. Results

The effects of elicitor treatments on various growth characters of chickpea and maize plants were recorded in Tables 1 and 2. Statistical analysis of the data showed that these treatments had significant ($p = 0.01$) effect on all the growth characters of chickpea (Table 1) except T_2 that gave the non significant difference in the plant height. In case of maize plants, the three treatments significantly ($p = 0.05/0.01$)

Table 1 Effect of k-carrageenan elicitor treatments on various growth characters of chickpea plants.

	Growth characters				
	Plant height (cm)	No. of pods plant^{-1}	No. of branches plant^{-1}	No. of leaves plant^{-1}	Days to flowering
T_1	**80.37 ± 1.03	**76.23 ± 0.61	**25.00 ± 1.00	**125.60 ± 0.27	**37.00 ± 1.00
T_2	68.50 ± 1.08	**59.97 ± 1.27	**16.50 ± 0.30	**89.00 ± 1.00	**45.00 ± 1.00
Control	67.23 ± 1.53	55.77 ± 0.91	14.00 ± 1.00	81.00 ± 1.00	47.00 ± 1.00
T_3	**74.00 ± 1.00	**68.90 ± 0.17	**21.00 ± 3.61	**121.00 ± 1.00	**40.00 ± 1.00
Control	66.50 ± 0.10	57.00 ± 1.00	13.50 ± 0.10	88.33 ± 2.52	42.00 ± 1.00
LSD at $p = 0.05$	1.50	1.25	1.89	1.82	1.58
LSD at $p = 0.01$	1.85	1.54	2.34	2.26	1.95

*Significant at $p = 0.05$.
**Significant at $p = 0.01$.

Table 2 Effect of k-carrageenan elicitor treatments on various growth characters of maize plants.

	Growth characters				
	Plant height (cm)	No. of cobs plant^{-1}	Stem diameter (mm)	No. of leaves plant^{-1}	Days to flowering
T_1	**156.50 ± 1.00	1.22 ± 0.02	**21.70 ± 0.10	**20.00 ± 1.73	53.00 ± 1.73
T_2	**153.20 ± 0.20	1.21 ± .01	18.33 ± 0.21	14.00 ± 1.00	55.00 ± 4.36
Control	150.60 ± 0.56	1.20 ± 0.01	18.63 ± 0.25	16.00 ± 1.00	55.00 ± 1.00
T_3	*155.50 ± 1.00	1.28 ± 0.01	**19.40 ± 0.20	*17.67 ± 1.16	54.00 ± 3.61
Control	153.13 ± 0.40	1.30 ± 0.03	18.60 ± 0.10	15.00 ± 1.00	56.00 ± 5.29
LSD at $p = 0.05$	1.00	0.02	0.27	1.86	5.04
LSD at $p = 0.01$	1.94	0.05	0.53	3.62	9.83

*Significant at $p = 0.05$.
**Significant at $p = 0.01$.

affected the plant height whereas only T_1 and T_3 had significant ($p = 0.05/0.01$) effects on stem diameter and number of leaves plant^{-1}. Number of cobs plant^{-1} and days to flowering in maize plants did not significantly respond to the applied treatments.

Mean values for the plant height of chickpea and maize plants were found in the range of 66.5–80.3–150.6–156.5 cm, respectively. However, maximum plant heights in the two plants were recorded in T_1 treated plants followed by T_3 and T_2 treated plants as compared to controls. Similar trend was observed in the number of branches, leaves and pods plant^{-1} in chickpea. Stem diameter and number of leaves plant^{-1} for maize plants were maximum in T_1 treatment. Days to flowering for both plants showed that flowering started earlier in T_1 treated plants while late flowering was recorded in other plants.

Analysis of ISM_S in leaves, stem and grains of the plants (Table 3) indicated that elicitor treatments had significantly ($p = 0.01$) increased the ISM_S concentration in these components. In few cases the effect of these elicitor treatments was non significant i.e., in the T_2 (in maize leaves and grains of chickpea) and T_3 (in stem of chickpea) the ISM_S concentrations were less than the controls. This might be due to the physiological conditions of the related parts of the plants. In general, the ISM_S contents were high in T_1 and T_3 treated plants.

4. Discussion

In a series of elicitor activity experiments the elicitor preparation of k-carrageenan at a concentration of 100 μg glc eq ml^{-1} was found very effective and induced maximum browning and high level of secondary metabolites (disease resistance compounds) in various crop plants like chickpea, carrots and potatoes (Bi and Iqbal, 1999; Bi et al., 2008). This elicitor dilution was applied in the present field experiments and observed its effect on various growth characters of chickpea and maize plants. Mercier et al. (2001) also found 100 μg ml^{-1} and 1000 μg ml^{-1} concentrations of algal polysaccharide 'λ carrageenan' as an effective dilution for high induction of defense related genes in tobacco leaves. A recent review has described that the seaweed extracts can serve as an important source of plant defense elicitors (Khan et al., 2009).

The growth results of the chickpea plants revealed that liquid elicitor treatments T_1 and T_3 were found more effective than the T_2 treatment. The T_1 and T_3 treated plants were healthier and large sized, their leaves, stem and flowers were of bright colors and more fresh and grains were bigger in size than those found in the controls, as seen in the Fig. 1 while T_2 treatment did not show any pronounced effect on the overall growth of chickpea plants. Moreover the plant height and the number of pods plant^{-1} of chickpea were found in the same range as previously described for chickpea plants treated with phosphorus and farmyard manure fertilizers (Basir et al., 2005). This shows that the effect of k-carrageenan elicitor is more or less equal to that of fertilizers. Increase in plant height, number of leaves and other growth characters of *Arabidopsis thaliana* with the application of seaweed *Ascophyllum nodosum* extract has recently been reported (Rayorath et al., 2008).

Isoflavones and related pterocarpans (phenolics) constitute a group of secondary metabolites primarily in leguminous

Table 3 Estimation of Induced Secondary Metabolites (ISMs) in different components of chickpea and maize plants treated with k-carrageenan elicitor treatments.

	Chickpea		Maize
	Leaves	Stem	Leaves
First time analysis			
T_1	**1.2816 ± 0.0001	**11.0400 ± 0.0001	**0.2798 ± 0.0001
T_2	**0.4413 ± 0.0001	**5.8212 ± 0.0001	0.1202 ± 0.818
Control	0.4089 ± 0.0001	5.7994 ± 0.0001	0.1498 ± 0.0001
T_3	**1.2321 ± 0.0001	**7.9699 ± 0.0001	**0.2352 ± 0.0001
Control	1.0119 ± 0.0001	4.2358 ± 0.0001	0.1997 ± 0.0002
LSD at p = 0.05	0.00015	0.00016	0.0260
LSD at p = 0.01	0.00030	0.00019	0.0321

	Chickpea		Maize	
	Leaves	Stem	Grains	Leaves
Second time analysis				
T_1	**4.5418 ± 0.0001	**3.8631 ± 0.0001	**3.4571 ± 0.0001	**5.9654 ± 0.0001
T_2	**3.5820 ± 0.0001	**3.2096 ± 0.0001	2.0117 ± 0.0001	**5.1419 ± 0.0001
Control	3.1758 ± 0.0001	2.8931 ± 0.0001	2.1984 ± 0.0002	4.8781 ± 0.0001
T_3	**4.4902 ± 0.0001	3.0676 ± 0.0001	**3.0997 ± 0.0001	**4.8692 ± 0.0001
Control	3.4718 ± 0.0001	3.0823 ± 0.0001	2.4627 ± 0.0001	4.4464 ± 0.0001
LSD at p = 0.05	0.00016	0.00016	0.00017	0.00016
LSD at p = 0.01	0.00020	0.00020	0.00020	0.00020

Results were obtained by averaging triplicate samples.
*Significant at p = 0.05.
**Significant at p = 0.01.

plants and are known for their role as disease resistance compounds 'phytoalexins' (Barz and Welle, 1992). An earlier study has confirmed that seaweed elicitor applications increased the constitutive phenolic levels in plants (Jayaraj et al., 2008). In the present field trials of elicitor treatments, metabolites induced in the leaves and stems of the tested plants were estimated twice during the study. UV absorbances of the alcoholic extracts of control and treated samples were measured in the UV range of 190–400 nm. Absorption values were assumed to be proportional to the amount of ISM_S. The λ max of 260–270 nm referred to the metabolites induced in leaves, stem and grains of chickpea and maize plants. Mostly the ISMs concentrations were quite high in T_1 and T_3 treated plants as compared to controls as indicated by the mean values of ISMs (Table 3). This suggested that the better response of chickpea plants could be due to high accumulation of ISMs (phytoalexins) in various components of these plants because high ISMs induction strengthens the defense mechanism of the plants against diseases, thus limiting the pathogen attack which ultimately results in enhancement of plant protection (Darvill and Albersheim, 1984). On the other hand T_1 and T_3 treatments added a regular and smooth increase in the growth of plants. The non treated plants (controls) and some T_2 treated plants were thin, small sized and their leaves turned yellow especially the plants under T_2 treatment after 6 weeks of elicitation. This shows that these plants were less resistant to pathogen and collapsed earlier.

Figure 1 (A) Elicitor treated chickpea plants, (B) control chickpea plants.

It is obvious from the presented data that applying liquid elicitor around the sowing seeds in soil (T_1) is quite better than using it as a foliar spray (T_3) on plants. The reason could be that the T_1 preparation was absorbed largely into the soil and help in soil conditioning which thus helped in increasing the plant strength and growth. This study also supported the results reported earlier that liquid elicitor preparation of seaweeds enhanced the defense system of plants (Bi and Iqbal, 2008). In the case of maize plants T_1 treatment was found better than the other two treatments in increasing plant height, stem diameter, number of leaves plant^{-1} and ISMs induction in leaves. Days to flowering and number of cobs plant^{-1} were not significantly affected by these elicitor treatments. It may be possible that some other doses of elicitor are required for foliar spray (T_3) and for the application in soil as solid formulation (T_2). As response of maize plants towards these treatments was not very promising that is why grains ISMs of maize were not estimated in these experiments.

On the basis of present results, it is concluded that seaweed polysaccharide elicitor k-carrageenan of *H. musciformis* can be used as a potent plant protectant as well as the good growth promoting agent especially for the chickpea plants. As elicitors are effective in very small quantities such that only micrograms of elicitor are required to apply on crops, therefore, it is easy and economical to utilize the polysaccharides of marine resources for the production of potentially active elicitors in large quantity. The commercial production of k-carrageenan elicitor may also prove a good step towards organic farming and help in the production of pesticide contamination free crops.

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