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DISTRIBUTION OF LECTIN IN NEMATODE-INFESTED AND UNINFESTED ROOTS OF *HIBISCUS ESCULENTUS*

by
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Summary. *Hibiscus esculentus* infested with root-knot nematode, *Meloidogyne incognita*, contained lectin mostly in the galled regions of roots from which nematodes had been removed. The ungalled regions as well as galls with nematodes contained negligible amounts of lectin. Lectin in uninfested roots was randomly distributed and was as high in amount as in galled regions of roots without nematodes.

Lectins are carbohydrate binding proteins or glycoproteins of non-immune origin that have been isolated from a variety of animal and plant sources (Goldstein and Hayes, 1978). They are thought to be involved in various biological processes such as defense mechanisms, pathogenesis, symbiosis, cell recognition (Etzler, 1986). Several vegetable species infested with root-knot nematodes, *Meloidogyne incognita*, show a marked increase in proteins in their galled roots (Owens and Specht, 1966; Alam *et al.*, 1976; Sinhababu and Sukul, 1983; Das and Sukul, 1986). The protein content of the galled roots of lady's finger plants is directly proportional to the intensity of infestation with root-knot nematodes (Chatterjee and Sukul, 1981). Proteins in galled roots may include lectins as a result of host response to nematode infestation. The purpose of the present investigation was to ascertain the distibution of lectin in nematode-infested and uninfested roots and its presence in the nematode.

Materials and methods

Seeds of lady's finger (*Hibiscus esculentus* L.) were surface sterilized and sown, one seed per pot, in one hundred 32 cm diameter pots containing an autoclaved mixture of clay soil and composted manure (2:1, v/v). Fifty pots were inoculate when the plants were at the 4-leaf stage with *M. incognita* (Kofoid *et* White) Chitw. juveniles (600 ± 32/pot). The remaining fifty pots served as uninoculated controls. The pots were kept in a shade house (30 ± 2°C) and were irrigated when necessary. Forty days after inoculation, all the plants were uprooted. Roots of the inoculated plants were divided into ungalled and galled and the latter further divided into those with nematodes, and those from wich nematodes had been removed. Uninoculated roots served as the control. Female nematodes were obtained from other samples of infested roots by stirring comminuted roots in 50% sucrose solution (Pableo, 1981); no enzyme was used for this method of extraction of nematodes.

All four groups of root pieces (uninoculated and 3 groups of inoculated) as well as nematodes were tested for lectin by the haemagglutination assay. This was performed with a Takatsy microtitrator (Cooke engineering Co., Alexandria, Va, U.S.A.) using 25 µl loops and 25 µl of a 2% suspension of rabbit erythrocytes (2 ml packed erythrocytes diluted to 100 ml) (Kabat, 1967). Equal volumes (25 µl) of normal saline, erythrocyte suspension and the serially diluted agglutinin were mixed, incubated for 1 h and scored. The reciprocal of the highest dilution of the agglutinin that produced visible haemagglutination was taken as the titre. The specific activity was defined as the total haemagglutination titres or unit per mg protein. The specific activities of the four groups of roots were tested by the one way analysis of variance.

Inhibition of haemagglutination was performed by incubating diluted agglutinin with serial dilutions of each type of sugar and then mixing the solutions with 25 µl of 2% rabbit erythrocytes (Kabat, 1976).

The rabbit erythrocytes (5 ml vol) were fixed with glutaraldehyde (Monsigny *et al.*, 1979). The suspension of fixed erythrocytes in phosphate buffered saline (pH 7.1) was used as the affinity matrix for the purification of lectins from different parts of the roots.

Each batch of fresh root pieces was crushed and extracted with acetone three times to remove lipids and fi-

nally dried at room temperature. The acetone powder of each sample was extracted with 10 vol of 10 mM sodium phosphate buffer containing 150 mM NaCl with a pH of 7.1 (PBS). The suspension was kept overnight at 4°C with occasional shaking. The extract was collected by centrifugation at 200 × g for 15 min at room temperature and the residue was repeatedly extracted with PBS. The pooled extracts were tested for protein and a haemagglutination activity. Protein content was estimated by the method of Lowry *et al.* (1951). An aliquot of 2 ml of the crude extract was incubated overnight with 2 ml of fixed rabbit erythrocytes at 4°C with occasional stirring. Then it was centrifuged at 10°C at 200 × g for 10 min. The erythrocytes were washed thoroughly with PBS until the absorbance at 280 nm was 0.02. The bound protein was eluted by incubating the erythrocytes with 50 mM glycine-HCl buffer (pH 2.3). The eluted material was neutralised, dialysed against PBS (pH 7.1) and concentrated.

The female nematodes in saline were sonicated using a Labsonic 2000 with an output power of -064 KHZ after one min. Sonication was done for 5 min with a 5-sec. interval at every 15 sec. The sonicated material was centrifuged 4 times at 200 × g each time for 15 min. All the clear supernatans were pooled and concentrated. This nematode extract was tested for protein and haemagglutination activity.

Results and discussion

Lectin was present in all the groups of roots but not in the nematode extract which contained only a very small amount of protein (0.013%). The specific activities of lectins in galled portions of roots without nematodes and uninoculated roots were high ($P < 0.05$) and of similar values (Table I). The specific activities decreased in galled regions with nematodes as well as in ungalled portions of infested roots. Ungalled regions did not contain any nematodes. Table II presents the specific activities of purified lectins and the degree of overall purification of lectins achieved. The specific activities of the four groups of roots were similar to those of the crude extracts (Table II). The purification fold was maximum in case of galled regions without nematodes. All the purified lectins were inhibited by *D*-mannose and not by *D*-galactose. Only in galled roots was *D*-xylose a better inhibitor than *D*-mannose (Table III).

The results show that lectin is present in the roots of both nematode-infested and uninfested plants. It is unequally distributed in nematode-infested roots *vis-a-vis* uninfested ones. The marked increase in the specific activity of lectin in galled roots without nematodes as compared to those with nematodes may be due to the binding of lectins to the carbohydrates released by crushing in the latter case, and similarly this may explain the absence of lectin activity in nematodes alone. Carbohydrates were detected in the stylet exudate from living females of *Meloidogyne* sp. (Bird, 1968) and on the surface of *M. incognita* (McClure

TABLE I - *Specific activities of lectins from the crude extracts of the nematode-infested and uninfested roots of* Hibiscus esculentus.

Fractions	Total volume (ml)	Total protein (mg/ml)	Total titre	Specific activity (Titre/mg)[a]
C[b]	10	0.36	640	175.3[a]
G	12	0.63	192	25.4[b]
UNG	11	0.49	352	65.3[c]
GWN	10	0.26	320	120.7[d]

[a] Data followed by different small letters in the column indicate significant difference ($P<0.05$) by analysis of variance.
[b] C = Uninoculated roots; G = Galled roots; UNG = Ungalled portion of infested roots; GWN = Galled roots without nematodes.

TABLE II - *Specific activities of the purified lectins from the nematode-infested and uninfested roots of* Hibiscus esculentus.

Fractions	Total volume (ml)	Total protein (mg/ml)	Total titre	Specific activity (Titre/mg)[a]	Purification (fold)[b]
C[c]	2	0.05	512	5120.0[a]	29.2
G	2	0.21	128	340.8[b]	12.0
UNG	1.5	0.35	256	487.6[c]	7.4
GWN	2	0.03	256	4266.7[d]	35.3

[a] Data followed by different small letters in the column indicate significant difference ($P<0.05$) by analysis of variance.
[b] For each fraction, purification fold is obtained by dividing the specific activity of purified fraction by that of crude fraction from Table I.
[c] C = Uninoculated roots; G = Galled roots; UNG = Ungalled portion of infested roots; GWN = Galled roots without nematodes.

TABLE III - *Inhibition by various sugars of the haemagglutinating activity of purified lectins from the nematode-infested and uninfested roots of* Hibiscus esculentus.

Sugars	Fractions[a]	Minimum concentration required for complete inhibition of purified lectin (mM)
D-Mannose	C[b]	3
	G	55
	UNG	13
	GWN	13
D-Galactose	C	>111
	G	>111
	UNG	>111
	GWN	>111
D-Xylose	C	>135
	G	16
	UNG	>135
	GWN	>135

[a] Concentrations of proteins used for the fractions C, G, UNG and GWN were 0.5, 5.25, 8.75 µg, respectively.
[b] C = Uninoculated roots; G = Galled roots; UNG = Ungalled portion of infested roots; GWN = Galled roots without nematodes.

and Zuckerman, 1982). The low specific activity of the ungalled portions of infested roots might be due to the inhibition of synthesis of lectin in these regions or the translocation of lectin from this region to the galled region. Further experimentation is required before conclusions can be made on the significance of lectin accumulation in the galled regions of roots.

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