

## Potential of Legume Lectins as Antagonistic Biomolecules to Root Knot Nematode, *Meloidogyne incognita* in Tomato

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### Abstract

Lectins from pigeonpea, and chickpea were extracted from their mature seeds and evaluated their potential use as biomolecules antagonistic to root knot nematode, *Meloidogyne incognita* on tomato in a phytotron providing 25 °C day and 20 °C night temperature with 12 h photoperiod. Exposure of egg masses of *M. incognita* to a concentration of 100 µg/ml lectin protein of chickpea and pigeonpea, haemagglutination activity of 5 and 12 units respectively, reduced hatching of second stage juveniles by 15 to 29%, adversely affected mobility of the hatched juveniles and restricted their invasion into roots by 78 to 81%. The fecundity and the soil and root population density of *M. incognita* were also adversely affected as recorded at day 90, ultimately reducing the hazard indices measured in terms of root galling of tomato. Analysis of lectins in different wild relatives of pigeonpea showed genotypic differences in the levels at different stages of pod development.

**Keywords:** Biomolecule, legume lectins, chickpea, pigeonpea, *Lycopersicon esculentum*, *Meloidogyne incognita*

### Introduction

Lectins are carbohydrate-binding proteins of non-immune origin that recognize and interact specifically and reversibly with oligosaccharide chains of glycoconjugates without altering the covalent structures of any of the recognized glycosyl ligands (Goldstein *et al.*, 1980; Goldstein and Pretz, 1986). They are present in both animals and plants and particularly abundant in the family Fabaceae (Van Driessche, 1988). Seeds are the richest source of lectins (Sharon and Lis, 1990) constituting 10% of the total seed proteins located in the cotyledons. Other plant parts such as leaves, barks and roots also contain smaller amounts of lectins (Etzler, 1986; Sharma and Salahuddin, 1993). The presence of lectins in a protein mixture can easily be assessed by agglutination of erythrocytes (Van Driessche, 1988). Lectins have been implicated in plant defense against bacteria, fungi, insects, and nematodes (Borgonie *et al.*, 1982; Forrest *et al.*, 1989; Czapla and Lang, 1990; Marban-Mandoza *et al.*, 1992) and even speculated to confer along with protease inhibitors a broad-spectrum nematode resistance by gene pyramiding in transgenic plants (Burrows *et al.*, 1998).

Some lectins such as Concanavalin A (Con A), soybean

agglutinin (SBA), wheat germ agglutinin (WGA), *Lotus tetragonolobus* agglutinin (LOT), *Limax flavus* agglutinin (LFA), and *Limulus polyphemus* agglutinin (LPA) have shown nematicidal activity against root-knot nematodes (*Meloidogyne* spp.) (Davis *et al.*, 1989 a&b; Marban-Mandoza *et al.*, 1992). Chickpea and pigeonpea are important crops plants in semi-arid tropics and are known to contain lectins within their seeds similar to other legumes. The present study was aimed to isolate and evaluate the effect of chickpea and pigeonpea lectins from mature seeds on egg hatch, juvenile invasion into roots, reproduction and population density of *M. incognita* on tomato. We also evaluated the genotypic difference in lectin levels from developing pods of pigeonpea.

### Materials and methods

#### Extraction of lectins from mature seeds

The lectins were extracted from dry seeds of chickpea and pigeonpea as described earlier by Kolberg *et al.*, (1983) with some modifications. Seed (10 g) were soaked in distilled water, allowed to swell overnight at 4 °C. The swollen samples were extracted in 50 ml of saline solution (0.5M sodium chloride, 20 mM calcium chloride, 20 mM

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manganese chloride and 0.2% sodium azide) by homogenization, stirred the suspension overnight in cold, filtered through cheese cloth and centrifuged at 12000 rpm for 30 min at 4 °C. The supernatant was precipitated with 60% saturated ammonium sulfate solution and resuspended the precipitate in saline buffer and dialyzed overnight at 4 °C against saline solution to remove the ammonium sulfate. The concentrated lectin is used for haemagglutination assay, protein estimation and nematicidal activity.

### **Extraction of lectins at different stages of pod development in wild and cultivated genotypes of pigeonpea**

The lectins were extracted separately from leaves (10 to 15 days after flowering), immature pods (20 to 25 days after flowering) and mature pods (30 to 36 days after flowering) of wild genotypes of pigeonpea (*C. acutifolius* and *C. scarabaeoides*). One g of each sample was extracted in 5 ml of 0.5 M phosphate buffer (0.5 M potassium phosphate buffer pH 7.2, 20 mM calcium chloride, 20 mM manganese chloride and 0.2% sodium azide) by homogenization, stirred the suspension overnight in cold, filtered through cheese cloth and centrifuged at 12000 rpm for 30 min at 4 °C. The supernatant was collected and used directly for haemagglutination assay (Van Driessche, 1988) and quantification of protein (Bradford, 1976).

### **Haemagglutination assay**

Lectin activity was assessed by haemagglutination assay using trypsinized rabbit erythrocytes as described by Van Driessche (1988). Rabbit blood was collected in equal volume of Alsever solution (2.05 g glucose, 0.80 g sodium citrate and 0.42 g sodium chloride in 100 ml distilled water, pH 7.2) containing few drops of Heparin. The red blood cells were collected by centrifugation at 1500 rpm for 10 min, washed and diluted with saline (0.9% sodium chloride, 0.02% sodium azide, pH 6.5) to yield a 4% (v/v) suspension that was incubated with trypsin (1 mg trypsin per 100 ml diluted erythrocytes) for 30 min at 25 °C. Lectin samples were serially diluted two-fold with equal volume of saline to 50 µl in a microtiter plate. Trypsinized rabbit erythrocytes (50 µl) were added to each dilution and incubated for 2 h at room temperature. Haemagglutination units (H.U.) were calculated as described earlier (Grant *et al.*, 1983).

### **Quantification of protein**

The total protein (mg/ml) in the lectin samples was estimated by Bradford's method (Bradford, 1976) using bovine serum albumin protein concentrations in the range of 0.1 to 1.0 mg/ml as standard.

### **Nematicidal activity**

The effect of lectins on nematodes was assessed in an experiment conducted in a randomized block design with five treatments and ten replications in terms of egg hatching, mobility, root invasion by second stage juveniles (J2), fecundity of the nematode and nematode populations in soil and root galling on tomato.

### **Effect on egg hatch**

To study the effect of lectins on egg hatch, 40 egg masses of *M. incognita* were handpicked from roots of eggplant (*Solanum melongena* L.). The egg masses were placed in three ml aqueous solution containing lectins at 100 µg protein/ml in 2.5 cm diameter sterile petri dishes. These egg masses were incubated for a period of seven days at 28±1 °C. The J2 that hatched in seven days were counted.

### **Effect on root invasion**

The emerged J2 in different treatments were inoculated to the rhizosphere of 21 day-old tomato (*Lycopersicon esculentum* var. Pusa Ruby) seedlings at the rate of 2 J2/seedling grown in plastic pots (5 cm and 15 cm diameter) containing 100 and 500 cm<sup>3</sup> soil and sand mixture (1:3), respectively. The pots were maintained in a phytotron providing 25 °C day and 20 °C night temperature with 12 h photoperiod. The plants were irrigated daily twice with Hoagland nutrient solution. Invasion of J2 in roots of tomato was observed 15 days after inoculation in 5 cm diameter pots (Byrd *et al.*, 1983). The experiments were repeated twice.

### **Effect on root galling**

Root galling was recorded at 90 d of inoculation in 15 cm diameter pots (500 cm<sup>3</sup> soil and sand mixture) and gall index was estimated following standard procedures (Marban-Mandoza *et al.*, 1992).

### **Effect on fecundity**

To observe fecundity, ten egg masses were handpicked after 90 d of treatment and separated the eggs in 0.5 ml of 0.5% sodium hypochlorite in glass-stoppered test tubes, diluted to 50 ml and a 3 ml aliquot counted for the number of eggs under a stereo binocular microscope.

### **Effect on soil and root populations**

Soil and root populations were monitored after 90 d of treatment. To assay the nematode population in soil, 200 cc of pot soil was processed by Cobb's modified shifting and gravity technique. For determining the total root populations, 90 d-old roots were washed free of adhering soil and sand, cut into small pieces and the whole root system was

macerated with water in a blender for 30-40 sec. The root suspension was collected in a beaker and sieved through a coarse sieve to separate the debris and the final volume was made up to 100 ml. An aliquot of 5 ml was examined under a stereoscopic binocular microscope.

### Statistical analysis

The data on egg hatch, mobility of J2 and invasion of J2 in roots, fecundity, nematode population density in soil, and root galling in all the five treatments including control (without lectin) were statistically analyzed for variance. The means were compared by using Fisher's least significant difference test (LSD of CD).

### Results and discussion

Lectin content of mature seeds, measured as H. U., was higher in pigeonpea than chickpea (Table 1). Lectin levels were assayed in leaves and in developing pods for all the selected pigeonpea cultivars (Table 2). Lectin levels were not detectable or very low in the leaves of most of the cultivars (data not shown). Lectin levels decreased with increasing stages of pod development (Table 2). Two of the wild species belonging to *C. acutifolius*, ICPW1 and ICPW2 showed significantly higher levels of lectin in contrast to those in *C. scarabaeoides*, ICPW 83 and ICPW90. The other four wild species belonging to *C. scarabaeoides* also showed low levels of lectin (Table 2). Lectin levels were low in the

**Table 1. Total protein content and lectin haemagglutination activity of mature seeds**

| Lectin samples | Total protein                  |                         | Calculated H.U.* (per mg protein) |
|----------------|--------------------------------|-------------------------|-----------------------------------|
|                | content (mg ml <sup>-1</sup> ) | Haemagglutination titre |                                   |
| Chickpea       | 6.1                            | 1:16                    | 52                                |
| Pigeonpea      | 2.5                            | 1:16                    | 128                               |

\* H.U. - Haemagglutination units

developing pods of the cultivated pigeonpea, (ICPL87 and ICPL85010), similar to the wild species *C. scarabaeoides* and were almost not detectable in the mature pods. The species *C. scarabaeoides* are easily crossable with the cultivated *C. cajan* than *C. acutifolius* that could play a role in selecting species with high lectin levels for nematocidal activities. In the wild genotypes of pigeonpea, the lectin levels declined with seed maturity (Table 2) where as the lectin levels in cultivated species were lower than that in the some wild species.

The lectin present in 100 µg ml<sup>-1</sup> of protein reduced the egg hatch (Table 3). The reduction in egg hatch was higher in pigeonpea lectins (29%; 12 H.U.) than chickpea (15%; 6 H.U.) lectins. The treatments influenced the mobility of the hatched juveniles and also adversely affected the invasion

**Table 2. Lectin levels at various stages of pod development in genotypes of *Cajanus acutifolius*, *C. scarabaeoides* and *C. cajan***

| Genotype/cultivar       | Total protein (mg ml <sup>-1</sup> ) |          |        | Haemagglutination titre in pods |          |        | Calculated H.U.* (per mg protein) |          |        |
|-------------------------|--------------------------------------|----------|--------|---------------------------------|----------|--------|-----------------------------------|----------|--------|
|                         | Juvenile                             | Immature | Mature | Juvenile                        | Immature | Mature | Juvenile                          | Immature | Mature |
| <i>C. acutifolius</i>   |                                      |          |        |                                 |          |        |                                   |          |        |
| ICPW1                   | 0.17                                 | 0.18     | 0.13   | 1:256                           | 1:64     | 1:32   | 30116                             | 7112     | 4924   |
| ICPW2                   | 0.21                                 | 0.16     | 0.20   | 1:128                           | 1:64     | 1:16   | 12190                             | 8000     | 1600   |
| <i>C. scarabaeoides</i> |                                      |          |        |                                 |          |        |                                   |          |        |
| ICPW 90                 | 0.07                                 | 0.10     | 0.20   | 1:16                            | 1:4      | 1:2    | 4572                              | 800      | 200    |
| ICPW 83                 | 0.14                                 | 0.10     | 0.20   | 1:8                             | 1:4      | 1:2    | 1142                              | 800      | 200    |
| ICP 15731               | 0.11                                 | 0.147    | ND     | 1:8                             | 1:4      | ND     | 1456                              | 544      | ND     |
| ICP 15695               | 0.074                                | 0.12     | ND     | 1:4                             | 1:2      | ND     | 1080                              | 334      | ND     |
| ICP 15738               | 0.068                                | 0.11     | ND     | 1:8                             | 1:4      | ND     | 2354                              | 728      | ND     |
| ICP15753                | 0.093                                | 0.065    | ND     | 1:16                            | 1:4      | ND     | 3440                              | 1230     | ND     |
| <i>C. cajan</i>         |                                      |          |        |                                 |          |        |                                   |          |        |
| ICPL87                  | 0.06                                 | 0.05     | 0.24   | 1:8                             | 1:2      | ND     | 2664                              | 800      | ND     |
| ICP 815753              | 0.05                                 | 0.033    | 0.124  | 1:16                            | 1:2      | ND     | 6400                              | 1212     | ND     |

\* H.U. - Haemagglutination units, ND - not detectable

**Table 3. Effect of lectins on hatching, mobility, invasion, fecundity, and reproduction of *Meloidogyne incognita* and root galling of tomato**

| Lectin Source      | Hatching of J2 (7days) | %immobility (7 days)** | % invasion (15 days)** | Fecundity* | Soil population 200 g soil (90 days)* | Root population (90 days)* | Gall index (90 days) |
|--------------------|------------------------|------------------------|------------------------|------------|---------------------------------------|----------------------------|----------------------|
| Pigeonpea          | 18.60                  | 12.78                  | 13.43                  | 16.73      | 36.88                                 | 16.58                      | 2.5                  |
| Chickpea           | 20.41                  | 8.32                   | 12.38                  | 16.79      | 28.28                                 | 19.74                      | 2.3                  |
| Control (- lectin) | 22.05                  | -7.90                  | 29.85                  | 21.17      | 59.26                                 | 46.03                      | 3.9                  |
| LSD (P<0.05)       | 0.29                   | 0.75                   | 0.82                   | 0.23       | 12.46                                 | 0.42                       |                      |

n= 10; \*Square root transformed values; \*\*angular transformed values [Protein conc. =100 µg/ml; Phytotron: temp. °C day/night (25/20), photoperiod day/night (12/12)]

of juveniles into tomato roots. Compared to control, 78 to 81% of the juveniles exposed to lectins for a period of 7 days did not invade the roots. The lectin from chickpea greatly reduced (81.5%) the invasion of J2 into the roots. The fecundity, soil and root population, and root gall index, as estimated 90 days after inoculation, were also adversely affected by lectin treatments. Compared to control, the number of eggs per egg mass was 37% in pigeonpea and 30% in chickpea lectin treatments. A similar trend was reflected in soil population reduced by 70% with both the lectins. The per cent reduction in root populations was 87% in pigeonpea and 82% in chickpea lectins. As a result of the reduction in fecundity and nematode multiplication in soil and root, the root infection measured as gall index, was much lower (2.3 to 2.5) compared to controls (3.9).

The inhibition in hatching and invasion of J2 into tomato roots to varying degrees in chickpea and pigeonpea lectin treatments was presumably due to differential binding of lectins with amphidial secretions and body surface of the nematode (Davis *et al.*, 1989a; McClure and Stynes, 1988; Spiegel *et al.*, 1982). There are perhaps, other features that affect the influence of lectins on nematode activity. For example, Con A, SBA, WGA and LOT lectins did not influence the invasion of *M. incognita* race 1 or *M. javanica* juveniles in soybean cultivars, Picket and Centennial, but the latter exhibited hypersensitive reaction to infection by *M. incognita* race 3 suggesting carbohydrate portion of glycoproteins to influence plant nematode reaction (Davis *et al.*, 1989b). These lectins increased the penetration of *Radopholus citrophilus* into citrus roots but heat denaturation of lectins reduced penetration (Kaplan and Davis, 1991). The mechanism for this *in planta* and *in vitro* effects remain obscure. A strong suppression of nematode reproduction was recorded in Centennial soybean when J2 of *M. incognita* (races 1 and 3) and *M. javanica* were incubated with Con A, SBA, WGA, LOT and *Limulus polyphemus* agglutinin, then added to the soil (Davis *et al.*, 1989b). The lectins, perhaps, impaired the clues for host finding, because of the specificity

of carbohydrate recognition in nematode-host interaction (Davis *et al.*, 1989 a & b) or they interfered with nematode sensory perception and therefore, to locate food or mate (Zuckerman, 1983). The suppression of root-knot nematode populations and reduction in root galling of tomato by Con A and PHA in *Arachis pintoii* and PHA in *Pueraria phaseoloide* (homologous to *Phaseolus vulgaris* lectin) was observed in tomato and tropical legumes (Marban-Mandoza *et al.*, 1992 & 1997). Further, it was found that Con A applied as soil drench reduced root galling caused by *M. incognita* in tomato roots (Marban-Mandoza *et al.*, 1997). The suppression of root knot nematode was maximum in chickpea and minimum in pigeonpea lectins. This could be due to the fact that unlike many legume lectins including BPA, Con A and pea lectins that are specific for mannose and glucose and showed little or no affinity for galactose, *Phaseolus mungo* lectin binds galactose and shows measurable but reduced affinity for N-acetyl galactosamine (Gatehouse *et al.*, 1993; Sharma and Salahuddin, 1993). Amongst the wild relatives of pigeonpea, genotypes ICPW 90 and ICPW 83 of *C. scarabaeoides* were earlier shown to possess fairly high degree of resistance to *Meloidogyne javanica* and *Rotylenchulus reniformis* (Sharma *et al.*, 1993). They observed differences in biological activity of the test lectins could be due to differential binding sites on nematode body and carbohydrate binding specificity (Borgonie *et al.*, 1987; Davis *et al.*, 1989a; Forrest *et al.*, 1989; Sharon and Lis, 1990; Sharma and Salahuddin, 1993; Strosberg *et al.*, 1986), specific amino acid residues and their modification through guanidation, carbamylation, succinylation (Means and Feeney, 1990), and even deglycosylation (Balasubramaniam *et al.*, 1993). The retention of toxicity even after deglycosylation might explain the reduction in fecundity of the nematode even after three months of treatment. It seems that the selective chemical derivatization of side chains of these lectins might provide invaluable prior information on the importance of specific amino acid residue for a particular biological activity. It is evident from the

present studies that chickpea and pigeonpea lectins have potential for use in the management of *M. incognita* on tomato and possibly other crops.

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