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Original Research Article

Study on protein and sugar content in *Meloidogyne incognita* infested roots of bitter gourd

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A B S T R A C T

Bitter gourd (*Momordica charantia*) is an economically important vegetable crop facing considerable yield loss due to infection by root-knot nematode (*Meloidogyne incognita)*. To understand the biochemical changes taking place during the early response to nematode attack in bitter gourd, a pot experiments were conducted. Nematodes were isolated and inoculated (500J2/pot) into the rhizosphere of seedlings. A rapid augmentation in the protein concentration in the first week and total sugar level in the fourth week of inoculation in infected roots was observed. The study indicates a higher rate of protein synthesis during invasion of nematodes for initiation of early primary resistance mechanism by the plants to combat nematode attack. Augmentation in sugar levels in the 4th week coincides with gall formation in the root tissue by the nematode and indicates greater influx of sugars in the attacked tissue for providing nutrition to the nematodes for their growth and survival.

Keywords

Momordica charantia, *Meloidogyne incognita*, Root-knot Nematode, Protein and Sugar, Rhizosphere

Introduction

Bitter gourd (*Momordica charantia*) is economically important vegetable in Southeast Asia. The fruit of bitter gourd has higher content in folate and vitamin C, while the vine tips are excellent sources of vitamin A. it also has medicinal value in the treatment of infectious diseases and especially diabetes as hypoglycemic agent. The root knot nematode is one of the important pests of bitter gourd and reported to cause 38 to 48.2% yield losses (Kaur and Pathak 2011). Among the root knot nematode species *Meloidogyne incognita* is major pest causes significant loss in quality and quantity of production and showed high pathogenic potential on bitter gourd (Chen and Tsay 2006; Anwar and McKenry 2010; Chandra et al. 2010; Singh *et al.*, 2012). Root-knot nematodes are obligate parasites, feeding on the cytoplasm of living plant cells, damaging them and cause root knot disease. Due to the root knot nematode infection, formation of typical root galls affect nutrients uptake and translocation of materials.

The plant is able to recognize the pathogen early in the infection process and leads to a rapid tissue necrosis at the site of infection, which is called the Hypersensitive Reaction (HR). It triggers inducible defense mechanisms includes- synthesis of phytoalexins and hydrolytic enzymes that attack fungi and bacteria, and alterations in the synthesis of cell-wall structural proteins (Lamb *et al.,* 1989). Many of these responses are due to transcriptional activation of specific genes that are collectively known as plant defense or defense-related genes (Mehdy, 1994) encode a variety of proteins including enzymes, regulatory and pathogenesis related (PR) proteins that control the expression of other defense related genes (Dixon *et al.,* 1994).

An induced defense protein makes the plant, resistant to pathogen invasion and has been correlated with defense mechanism (Rasmussen, 1991; Van Loon, 1997). Guozhong *et al.,* (2005) was showed that induction of polypeptides synthesis and enzyme mediated biosynthesis of antibiotic molecules control the activities of the nematode parasites during the early stage of infection. *M. incognita* infected soybean showed an increased protein and lipid metabolism (Simte and Dasgupta, 1987; Romabati and Dhanachand, 2000).

The major source of carbohydrate into nematode-induced feeding sites in *Arabidopsis thaliana* roots is sucrose and described as the main transported sugar in the phloem of this plant species (Haritatos *et al.,* 2000). During giant cell formation, sugar import mechanisms have been studied intensively in recent years (Juergensen *et al.,* 2003; Hoth *et al.,* 2005; Hammes *et al.,* 2005; Hofmann *et al.,* 2007, 2009, and 2010) and syncytia and giant cells showed significantly increased sucrose levels (Hofmann *et al.,* 2007; Baldacci-Cresp *et al.,* 2011). Changes in syncytial sugar levels may indicate altered sink strength and thus changes in systemic sugar partitioning. Sugar elevations were shown to contribute substantially to enhanced nematode development (Grundler *et al.,* 1991) and have major nutritional value for the obligate parasites. Cabello *et al.,* (2013) was showed the different transcript and sugar levels that were beneficial for nematode which affects the plant cells metabolism and nutrition. Further biochemical studies on bitter gourd against root-knot nematodes will be necessary to determine the pathogenecity and to understand involvement of sugar and proteins synthesis with their role during plant–nematode interactions.

The present study was carried out to study the biochemical changes taking place during the early stages of the disease process after initiation of infection, with reference to levels of total protein and sugar vis-a-vis control using standard techniques (Glick, 1957; Oser, 1965).

Materials and Methods

Samples of roots bearing galls along with their rhizospheric soil were collected under field conditions from vegetable farmlands of district Durg (Chhattisgarh state) using standard sampling methods and brought to the laboratory, in polythene bags. Isolation of the root knot nematodes was done by the Cobb's Sieving and decanting method and modified Baermann funnel technique (Southey, 1970). The Root knot nematode *Meloidogyne incognita* was identified microscopically by examining the perineal pattern of females and later confirmed from the Division of Nematology, IARI, New Delhi.

Pot experiments and laboratory analysis were next conducted in the net house and Parasitology lab of the School of Life Sciences, Pt. Ravishankar Shukla University, Raipur, to study the early biochemical changes in *Momordica charantia* in response to *M. incognita* attack. Seeds of the aforementioned plants were surface sterilized with 0.1% $HgCl_2$ solution and washed three times with sterile water.

After storage under moisture for two days they were then sown singly in disposable glasses along with coco pit powder and after a week, transferred into 10 cm. earthen pots holding 500 gm sterilized soil and farmyard manure (3:1). Larval populations 500 freshly hatched second stage juveniles (J2) were inoculated per pot into the rhizosphere of 15 day old seedlings at a depth of 3 cm. Regular watering of the plants was done until harvest.

In weekly intervals after inoculation of nematode, three replicates of each infected and three from control plants were carefully uprooted and washed for adhering soil and dried in an oven at 60ºC. Changes in total protein and total sugar levels were estimated by the methods (Lowry *et al.,* 1951) and (Yem and Willis, 1954) respectively. Statistical significance of the means was analyzed by ANOVA.

Results and Discussion

Estimation and analysis of early biochemical changes in the roots of infected Bitter Gourd plants vis-a-vis control in weekly intervals revealed the following features.

Changes in total protein

The results depicted in Table.1 and Fig.1. show that the percent increase in total protein content was maximum (105.26%) in the 1st week of infection in the infected roots compared from control which was found to rapidly decline by 32.97% in the 2nd week, followed by a subsequent decline of 15.51% in the 3rd week. In the 4th week however, the roots were found to tend to recover their protein content increasing gradually from 2.85% to a 21.15% hike in the 5th week.

Changes in total sugar

Initial changes in total sugar level (Table.2 and Fig.2) shows a 33.64% decline in 1st week after infection, followed by 10.61% decline in the 2nd week. It was found to increase gradually thereafter from 7.62% in the 3rd week being maximum (50.33 %) in the 4th week. On the contrary, in the 5th week the amount of sugar diminished to a negligible % hike of 0.3%.

Protein and Sugar contents exhibited highly significant ($F=8.838$, $P<0.001$) changes in infected plants compared from controls. However, a rapid augmentation in levels of protein was seen in roots of infected plants (Table.1 and fig.1) after 1st week of infection which speedily declined in 2nd week. Hofmann *et al.*, (2007) and (2008) and Nayak and Mohanty, (2010) agreed with the increase sugar and protein levels are due to high metabolic activity in diseased tissues.

Table.1 Concentration (mg/gm fresh weight) and % change in protein content in *Momordica charantia* root after nematode inoculation

WAI	Control	Infected	% Change
I	405.463 ± 0.025	832.266 ± 0.003	105.26
II	668.658 ± 0.007	448.143 ± 0.014	-32.97
III	825.153 ± 0.006	697.112 ± 0.003	-15.51
IV	746.906 ± 0.014	768.246 ± 0.002	2.85
V	369.896 ± 0.006	448.143 ± 0.011	21.15
F=8.838, P<0.001			

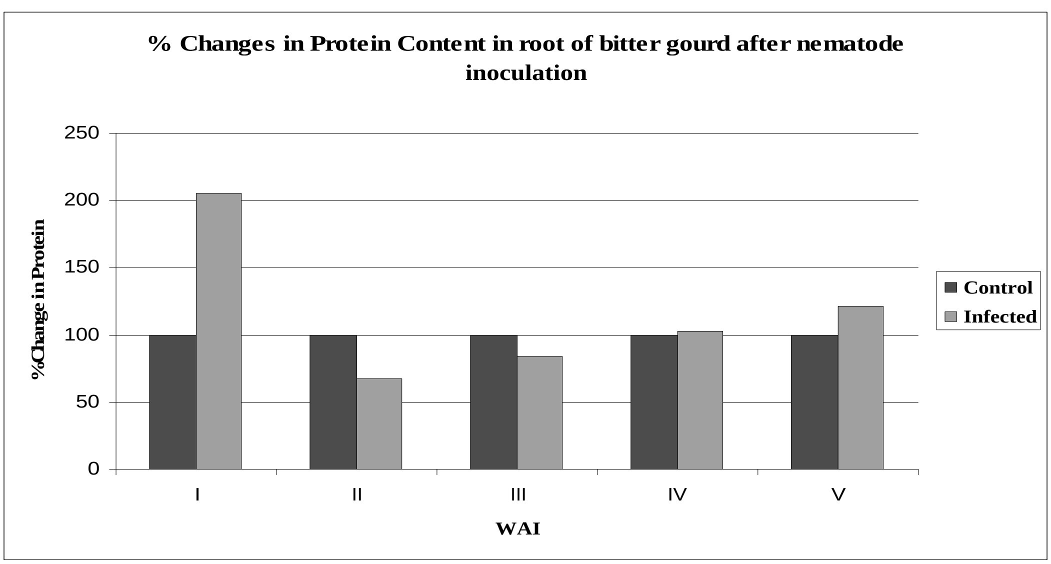
INDEX- WAI=weeks after inoculation

Table.2 Concentration (mg/gm fresh weight) and % change in sugar content in Bitter gourd root after nematode inoculation

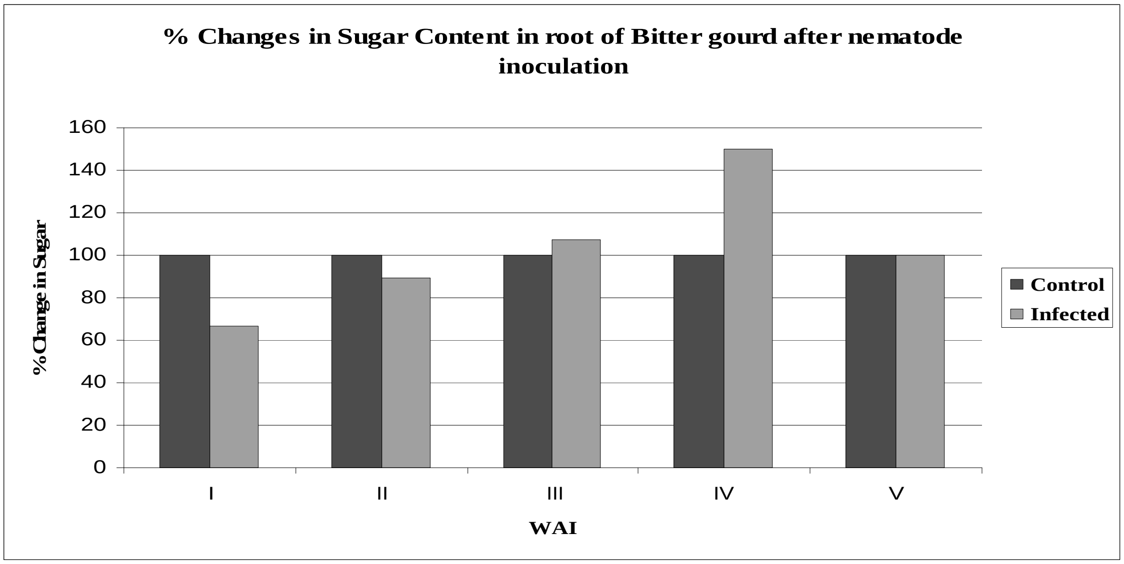
WAI	Control	Infected	% Change
I	70.609 ± 0.009	46.856 ± 0.038	-33.6409
II	42.413 ± 0.047	37.911 ± 0.035	-10.6145
III	34.949 ± 0.044	37.615 ± 0.019	7.627119
IV	35.542 ± 0.004	53.431 ± 0.036	50.33333
V	39.214 ± 0.036	39.333 ± 0.037	0.302115
F=8.838, P<0.001			

INDEX- WAI=weeks after inoculation

Figure. 1



Fugure.2



Increased levels of proteins, ascorbic acids, antibiotics, isozymes etc. have led to the concept of their possible role in defense reactions during pathogenesis (Uritani, 1971; Arrigoni, 1979; Jones, 1980; Ganguly and Das Gupta, 1981; Das gupta *et al.,* 1981; Premachandran, 1981). Abbasi *et al.,* (2008) also observed similar change that increases level of protein content as a result of inhibition of root-knot nematode infestation in okra and brinjal plants. Decreased level of proteins can be attributed to root-knot nematode induced gall formation and the development of giant cells that represent major sink for amino acids, which were imported into the roots *via* the vascular system (Hoth *et al.,* 2005). The susceptible green gram plant showed low protein content and high level of amino acids after inoculation of nematodes (Mohanty and Pradhan, 1989). After 30 days exposure to the nematodes (*M. javanica*) the protein content declined significantly in leaves of mung bean was observed by Ahmed *et al.,* (2009). The amino acid utilization by galls or giant cells reduces their availability for protein synthesis. In addition, amino acids were also probably formed due to the proteolysis of existing tissue proteins that essentially decreases the overall protein level.

A decline in the concentration of sugar in the initial stage of infection and its gradual increase up to 4th week after inoculation is possibly due to feeding behavior of nematode. Kannan (1968) reported as our result that the sugar values were lesser in the root-knot nematode infected *Acalypha indica* plant compared to the uninfected. Sijmons *et al.,* (1991) opined that the obligate plant parasitic cyst nematode *Heterodera schachtii* infects roots of *Arabidopsis thaliana* and induces syncytial feeding cells. The larvae release gland secretions by perforating one single cell with their stylet, which induce a dramatic re-organization of the tissue (Wyss, 1992). Several other changes take place along with syncytium formation leading to a rise in turgor pressure and drop in osmotic potential (Bo¨ckenhoff, 1995) depicting high metabolic activity.

The ability to induce feeding sites serves as an interface to orchestrate plant transport mechanisms and supply of nutrients in the appropriate quantity and quality to the growing larvae. Soluble carbohydrates are an essential source of energy. Presence of more golgi apparatus in the giant cells of galled roots is responsible for the increase in polysaccharide content in galled root exudates (Bird, 1961). Therefore; syncytial feeding sites contain high levels of sugars that can be taken up by the nematodes and the nematode feeding site shows most sugar pools were elevated and starch levels were higher in syncytia (Cabello *et al.*, 2013). Starch as an intermediate storage, compensate sugar levels and demands occur during the different phases of nematode feeding and development. Therefore, it is concluded that high sugar levels in 4^{th} week shows accumulation of starch later being degraded 5^{th} week onwards due to nematode development.

Earlier Vaitheeswaran *et al.*, (2011) studied that the pathogenesis impact was reflected with low sugar level, elevated protein and lipid levels and reduced total energy content in infected tissue of *Hibiscus cannabinus* with *M. incognita.* The results in the present work suggest that root-knot nematode infection induces increased protein concentration at initial stage of infection, which corresponds to resistance of nematode invasion during formation of root knots in infected plants.

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