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Estimation of starch content in resistant and susceptible varieties of cowpea (*Vigna unguiculata* L. Walp.) when inoculated with *Meloidogyne incognita*

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Abstract
The cowpea (*Vigna unguiculata* L. Walp.) is important pulse crop in India and recognised as vegetarian meat due to its immense nutrition al value. Phytoparasitic nematodes are the major constraints in the production of these pulses. Root knot nematode, *Meloidogyne incognita* is major nematode pest infecting pulses and cowpea is another host of this pest. Host Giant cells are formed by obligate parasite, *M. incognita* inside the host root that incites the physiological and biochemical changes in plant. In the present study pre-confirmed resistant and susceptible cultivar cowpea used as test plant to assess the starch changes in infected and non infected plants. The Starch content in both leaves and shoots showed a decrease from uninoculated to inoculated cowpea varieties. Higher reduction was observed in susceptible cultivars KM-5 and Gomati i.e. 34.74% and 31.33% respectively in leaves; 31.75% and 38.24% respectively in roots. Likewise, lowest reduction in starch i.e. 12.12% and 10.47% observed in resistant cowpea cultivar TVX-944 in leaves and shoot respectively over uninoculated plants. The result confirms earlier trend as reported by many workers. Narrow difference observed in starch content of resistant cultivar than susceptible cultivar. The study might helpful in further studies regarding phytoparasitic resistance cowpea varieties and their response toward parasites.

Keywords: *Meloidogyne incognita*, cowpea, starch

Introduction

Majority of Indian have vegetarian food habit hence, pulses are integral part of their diet due to their immense dietary value to supplement animal originated food and nutrition therein. The cowpea (*Vigna unguiculata* L. Walp.) is major cultivating pulse in India with excellent nutrient profile. It is also grown as green manure crop for soil improvement. Nutritionally valuable pulse production in India unfortunately suffers from several constraints remains hence, yield and total production remains deficient. Phytoparasitic nematodes have been recognized as one of the major constraint in pulse production in general and particular cowpea [18]. *Meloidogyne incognita* is a serious pest of cowpea, (*Vigna unguiculata* L. Walp.) [19] and causes 28.60 per cent losses. Many of workers reported nature of damage and losses caused by RKN in cowpea [15, 17]. In addition to yield loses the root knot nematode also responsible for the significant reduction in nodulation [2].

Understanding of host parasite interaction provides key knowledge for parasite management and bridge the yield gap. Root-knot nematodes are obligate parasites, feeding on the cytoplasm of living plant cells, by successfully established giant cell that serve as the sole nutrient source. The formation of feeding sites is accompanied by a massive solute import into giant cell. Sucrose is the major source of carbohydrate in giant cell incited by nematode in *Arabidopsis thaliana* roots. That also has been described as the main transported sugar in the phloem of this plant species [8]. During giant cell formation, sugar import mechanisms have been studied intensively in recent time [13, 12, 7, 9, 10, 11]. Elevation of sugar level is directly correlated with nematode development 6 due to its major nutritional value for the obligate parasites. Nematode feeds intermittently and stage specific on the cell. Third and fourth stage juveniles are comparatively short but non feeding stages. Starch which is buffer for sugar requirement in giant cell has been observed decreased in above ground parts and increased in roots by several Indian workers. Starch content of rice decreased with the increase in inoculum levels of *M. graminicola* as compared to uninoculated plants [4].

Same phenomenon has observed in the in green gram 16 where maximum reduction in starch content was reported in resistant cultivar of green gram as compare to susceptible cultivar.

The cultivar which able to afford need of parasites without undermining the own gives the stable yield. The aim of present study to estimate the starch content in resistant and susceptible cowpea cultivar infected with *M. incognita*.

Materials and Methods

Sterilized substrate of soil, sand in FYM mixture in the ratio of 2:1:1 used fill the disinfected earthen pot that interns used to grow the cowpea plants. Pre-confirmed population of *Meloidogyne incognita* were multiplied and maintained on a susceptible tomato variety (Pusa Ruby) used for infection test plants. Eggs were collected from the culture pot tomato plant roots and hatched J2s used for the inoculation.

Prescreened cowpea varieties (as resistant and susceptible) against *M. incognita* used for the experiment (Data not shown in the paper). For this experiment we selected [5] cultivars out of which Arka Samrudhi, IT-35956-1, TVX-944 are resistant and KM-5 and Gomati are susceptible. Sterilized and mixed soil culture filed in 1% formalin disinfected earthen pots @ 1 kg/ pot. The seeds were sown @ 4 to 5 seeds per pot. Each variety was replicated 3 times. Watering was done regularly after the emergence of seedlings. At 10 days after sowing the plants were thinned keeping one healthy seedling at the centre per pot. A small glass tube (2 cm long, 0.5 cm bore) was inserted into the soil near the rhizosphere of each of the surviving seedlings. Two weeks after seedling emergence axenised nematodes were counted under a stereoscopic microscope and released into the holes with the help of glass tube @ 1000 J2 per seedling in 10 ml. sterile water. For chemical analysis two sets of plants were maintained, one for the uninoculated control (Healthy) and the other infected with the nematode. Each set was arranged on separate platform in the green house in order to avoid cross infection.

One ml of starch extract was taken in a 100ml volumetric flask and diluted to 100ml with distilled water. 5 ml of the above extract was transferred in a 50 ml test tube. Then all the standards and sample test tubes were kept in ice bath for cooling, and 10ml of anthrone reagent was added to each test tube, allowing the reagent to run down the side of the flask. It was stirred slowly with a glass rod and then shaken thoroughly. The flask was kept in boiling water bath exactly for 7.5 minutes. Then the test tube was immediately cooled in ice-bath. After cooling, the O.D. at 630nm was measured and the starch content was calculated by the help of standard curve, which was multiplied by 0.91 to get the exact value of the same. Various observations recorded during the course of investigation were subjected to statistical analysis in a Completely Randomized Design. The comparison of the treatment means was done by calculating standard error of mean SE(m) and critical difference (C.D) in the following manner.

C.D. (critical Difference) = t at 5% for error d.f. × S.E (M) x square root 2

The difference between the two treatment means if greater than the CD value, it indicated the significant difference between the treatments.

Results and Discussion

Effect of *M. incognita* infection on starch content in leaves of cowpea varieties

Changes in the Starch content (%) in the leaves of resistant and susceptible cowpea varieties infested by Root Knot Nematode, *Meloidogyne incognita* is shown in table 1. Among the varieties under study Arka Samrudhi, IT-35956-1 and TVX-944 were found to show the resistance to *Meloidogyne incognita*. The Starch content showed a decrease from uninoculated to inoculated cowpea varieties. In case of resistant varieties Arka Samrudhi showed a decrease from 0.40 to 0.34 mg/g. similarly IT-35956-1 and TVX-944 showed a decrease from 0.24 to 0.19 mg/g and 0.33 to 0.29 mg/g, respectively. Further KM-5 and Gomati were found to show the susceptibility to *Meloidogyne incognita*. The Starch content showed a decrease from uninoculated to inoculated cowpea varieties. KM-5 showed a decrease in the value from 0.32 to 0.21 mg/g while Gomati showed a decrease in the value from 0.28 to 0.19 mg/g. Similarly, the percentage reduction was calculated for different resistant and susceptible cowpea varieties. Susceptible variety showed KM-5 the highest reduction (34.74%) followed by Gomati (31.33%). However the lowest reduction (%) was observed in TVX-944 (12.12%) followed by Arka Samrudhi (14.88%) and IT-35956-1 (21.13%). Similar trend was observed in green gram and bitter gourd resistant and susceptible cultivars when inoculated with *M. incognita*. 16, 14. Same phenomenon also been observed in rice when rice plant inoculated with *M. graminicola* 4. The reduction in starch content in shoot might be due to nutrient sink towards the giant cell formed by *M. incognita* where demand for sugar increases as infection increases. Difference of reduction in resistant and susceptible is directly correlates the starch sink with amount of infection.

Effect of *M. incognita* infection on starch content in root of cowpea varieties

Changes in the Starch content (%) in the root of resistant and susceptible cowpea varieties infested by root knot nematode, *Meloidogyne incognita* is shown in table 1. Among the varieties under study Arka Samrudhi, IT-35956-1 and TVX-944 were found to show the resistance to *Meloidogyne incognita*. The Starch content showed decrease from uninoculated to inoculated cowpea varieties. In case of resistant varieties Arka Samrudhi showed a decrease from 0.45 to 0.37 mg/g. similarly IT-35956-1 and TVX-944 showed a decrease from 0.42 to 0.35 mg/g and 0.29 to 0.26 mg/g, respectively. Further, KM-5 and Gomati were found to show the susceptibility to *Meloidogyne incognita*. KM-5 showed decrease in the value from 0.21 to 0.14 mg/g while Gomati showed decrease in the value from 0.34 to 0.21 mg/g. Similarly, the percentage reduction was calculated for different resistant and susceptible cowpea varieties. Susceptible varieties showed a highest reduction Gomati (38.24%) followed by KM-5 (31.75%). However the lowest reduction (%) was observed in TVX-944 (10.47%) followed by IT-35956-1 (17.32%) and Arka Samrudhi (18.52%). Similar trend was observed in green gram and bitter gourd resistant and susceptible cultivars when inoculated with *M. incognita* 16, 14. Amount of total carbohydrate was more in the susceptible banana clones than the resistant clones due to the burrowing nematode, *Radopholus similis* 5. Nutrient sink initiated due to infection *M. incognita* responsible for the accumulation of starch in the root. Inoculated susceptible cultivar roots have highest in reduction in starch content over uninoculated and shown similar trend as per shoot starch content. However, presence of more Golgi apparatus in the giant cells of galled roots is responsible for the increase in polysaccharide content in galled root exudates 1. Hence, 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 3. Syncytia induced by *Heterodera schachtii* in *Arabidopsis thaliana* found to be accumulated with starch that probably utilized as buffer stock for sugar 10. Plants roots we harvested for starch estimation was about to complete the two life cycles of parasite on it and hence it might be strong reason that accumulated starch could have utilized and hence composition is exhausted.

Table 1: Change in Starch content (mg/g) of resistant and susceptible cowpea varieties infected by Root knot Nematode, *Meloidogyne incognita*

SI. No.	Varieties	Starch content (mg/g fresh weight)				Starch content (mg/g fresh weight)			
		Leaf			% Decrease over Uninoculated	Root			% Decrease over Uninoculated
		Inoculated	Uninoculated	Mean		Inoculated	Uninoculated	Mean	
1	Arka Samrudhi (R)	0.34	0.40	0.37	-14.88	0.37	0.45	0.41	-18.52
2	IT-35956-1(R)	0.19	0.24	0.21	-21.13	0.35	0.42	0.39	-17.32
3	TVX-944(R)	0.29	0.33	0.31	-12.12	0.26	0.29	0.27	-10.47
4	KM-5(S)	0.21	0.32	0.26	-34.74	0.14	0.21	0.18	-31.75
5	Gomati (S)	0.19	0.28	0.23	-31.33	0.21	0.34	0.28	-38.24
	SEm (±)	0.02	0.02			0.02	0.03		
	CD(0.05)	0.05	0.05			0.05	0.08		

(+) - Increase, (-) - Decrease, (R) - Resistant, (S) - Susceptible

Conclusion
Cowpea cultivar KM-5 and Gomati having susceptible response against *M. incognita* shown remarkable reduction in starch content than resistant cultivar Arka Samrudhi, IT-35956-1 and TVX-944. More difference in starch content in uninoculated and inoculated plant is directly proportional to the infection. The resistant varieties shown less in difference between starch content hence able to strike balance between own requirement and parasitic demand. Studies on these cowpea varieties further might be useful for understanding the advanced mechanism of plant resistant.

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