Research Article

Chlorophyll Estimation through Biochemical Analysis in Resistant and Susceptible Cultivars of Cowpea against Infection by Root-Knot Nematode, *Meloidogyne Incognita*

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Abstract

Plants adapt themselves to overcome adverse environmental conditions, and this involves a cellular activities. Physiological experiments or metabolic profiling can quantify this response. Among several diseases of cowpea, root-knot nematode infection caused by *Meloidogyne incognita* causes severe damage to the plant and hence, the oil production. In the present study, we identified *M. incognita* morphologically and physiologically changes in cowpea. *M. incognita* was artificially inoculated at different levels of second stage juveniles (J2) to examine the effect on cowpea plant growth parameters. Chlorophyll content in *M. incognita* was also evaluated in response to infection. The results have demonstrated that nematode infestation leads to highest decreased by 97.44%, 74.33% and 93.42% chlorophyll content 'a', 'b' and chlorophyll (a+b) in Gomati in the leaves of the cowpea plants.

Keywords:cowpea, *M. incognita*, chlorophyll, resistant and susceptible

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Introduction

India is the world's largest producer and consumer of a wide variety of pulses which is dominated by tropical and sub-tropical crops. The carbohydrates provided by pulses are released slowly as compared to cereals and so have a high value for maintaining optimal blood sugar levels and restoring energy over a long period of time after the meals. The cowpea (*Vigna unguiculata* L.walp.) belongs to family Leguminaceae with subfamily Papilionaceae. Cowpea is called as vegetable meat due to high amount of protein with better biological value on duty weight basis. Cowpea is a leguminous crop and can fix atmospheric 'N' with the help of rhizobium bacteria. Deficiency of N, P and K are among major constraints on higher crop productivity in tropical regions. Hence for optimum yield, crop need to be fertilized properly.

Among the various pests, *M. incognita* poses a potential threat to the cultivation of pulse crops by infecting upon severe yield losses [8, 9, 10]. Physiological and biochemical activities of the host plant are drastically affected on nematode infection [2].

Leaf pigment composition is sensitive to plant stress and nematode infection causes either a loss of photosynthetic pigments (e.g. chlorophylls) or higher levels of photoprotective pigments, such as zeaxanthin or β-carotene [4]. Various forms of abiotic and biotic stresses damage plant leaf tissue and the chloroplasts [7]. The chlorophyll released from damaged chloroplasts has to be degraded rapidly to avoid cellular damage owing to its high reactivity [11]. Failure to degrade the chlorophyll may cause an accumulation of reactive oxygen species (ROS) that can easily damage the cellular organelles [5, 13]. That is why chlorophyll must be degraded rapidly following pathogen attack [6].

In our experiments, more chlorophyll a was degraded than chlorophyll b. This may have happened because chlorophyll a was degraded before chlorophyll b. This is in agreement with suggestions that the chlorophyll catabolic pathway, and specifically the enzyme chlorophyllase, is involved in modulating the plant defense response by affecting damage-derived photodynamic free chlorophyll levels, leading to a rise in ROS [6].

The production of cowpea crops unfortunately suffers from several constraints of which pest and disease are the most important once. Among pests, phytoparasitic nematodes have been recognized as one of the major constraint in pulse production. The extent of losses due to nematodes in cowpea crops is yet to be estimated properly but in cowpea production is estimated to cause annual yield losses of nearly 15% worldwide. In India average loss caused by root-knot nematode on pulses may be 14.6% which could go as high as 50-80% in some crops. In another study estimated 28.60 per cent losses due to root-knot nematode, *M. incognita* in cowpea.

Materials and Methods

Estimation of Chlorophyll content in leaves

150 mg leaf portion of each treatment were cut from the composite leaves and were immersed in 50 ml of 80 % acetone in a conical flask and kept in dark for 24 hours for extraction of chlorophyll from the leaf samples. Thereafter, the chlorophyll extracts were filtered through Whatman No.1 filter paper. Absorbance of the chlorophyll extract was measured at 645 nm and 663 nm using a colorimeter. The amount of chlorophyll-a, chlorophyll-b and total chlorophyll were calculated in mg/g fresh weight according to the following equations.

I. Chlorophyll –a (mg/g fresh weight of leaf) $= \{12.7(D-663) - 2.69(D-645)\} \times \frac{V}{1000 \times W}$

II. Chlorophyll-b (mg/g fresh weight of leaf) = $\{22.9(D\text{-}645) - 4.68\ (D\text{-}663)\} \times \frac{V}{1000 \times W}$

III. Total chlorophyll (mg/g fresh wt. of leaf) = $\{20.2\ (D\text{-}645) + 8.02\ x\ (D\text{-}663)\} \times \frac{V}{1000 \times W}$

Where
D –645 = Optical density at 645 nm
D-663 = Optical density at 663 nm
V = Final volume of 80 % acetone chlorophyll extract in ml
W = Fresh weight in g of corresponding amount of fresh leaves used in the extraction of chlorophyll

Result and Discussion

Effect of the nematode infection on chlorophyll content in leaves

Chlorophyll a

Chlorophyll a, b and total chlorophyll content were studied in nematode infested plant. A substantial reduction in chlorophyll a, b and total chlorophyll were noticed in *M. incognita* infested resistant and susceptible cowpea varieties. The results were presented in Table 3 and revealed that under inoculated and uninoculated resistant and susceptible cowpea varieties at 45 days of harvest.

Table 1 Change in chlorophyll 'a' content (mg/g) of resistant and susceptible cowpea varieties due to infection of root-knot nematode,*Meloidogyne incognita*

SI NO.	Varieties	Chlorophyll content (mg/g) in leaves			
		Leaves			% increase(+)/Decrease(-) over uninoculated
		Inoculated	Uninoculated	mean	
1	Arka variety(R)	0.95	1.17	1.06	-18.80
2	IT-3596-1(R)	1.12	1.22	1.17	-8.20
3	TVX-944(R)	0.89	1.04	0.97	-14.42
4	KM-5(S)	0.03	0.82	0.43	-96.34
5	Gomati(S)	0.02	0.78	0.40	-97.44
	SE(M)±	0.05	0.07		
	CD(0.05)	0.12	0.20		
Resistant(R), Susceptible(S)					

The present study (**Table 1**) was carried out for five varieties *viz.*, Arka variety, IT-35956-1- variety, TVX-944 variety, KM-5 and Gomati. Among these Arka variety, IT- variety and TVX-944 variety were found to be resistance to *M. incognita.* Chlorophyll 'a' content in these inoculated resistance varieties was found to be reduced from 1.17 mg/g to 0.95 mg/g, 1.22 mg/g to 1.12 mg/g, and 1.04 mg/g to 0.89 mg/g respectively whereas KM-5 and Gomati showed susceptibility with values of 0.82 mg/g to 0.03 mg/g and 0.78 mg/g to 0.02 mg/g, respectively.

Among the susceptible varieties Gomati showed difference between inoculated and uninoculated cowpea varieties which was found to be noticed highest reduction per cent of chlorophyll "a" content in Gomati (97.44) followed by

KM-5 (96.34). Among the resistant varieties there is decreased in chl a content of (18.84%) in Arka variety, (14.42%) in TVX-944 and (8.20) in IT-35956-1, respectively in comparison to uninoculated crop.

Among the susceptible varieties Gomati showed difference between inoculated and uninoculated cowpea varieties which was found to be noticed highest reduction per cent of chlorophyll "a" content in Gomati (97.44%) followed by KM-5 (96.34%). Among the resistant varieties there is decreased in chl a content of 18.84% in Arka variety, 14.42% in TVX-944 variety and 8.20% in IT-3596-1, respectively in comparison to uninoculated crop. (Table 1) due to chlorophyll "a" is identified as necessary pigment which converts light energy into chemical energy which helps to absorb the light. Chlorophyll molecule has mg^{2+} at center which makes it ionic and hydrophilic and the ring hydrophobic in nature. Results are in similar with the earlier findings [12, 1].

Chlorophyll "b"

The present study (**Table 2**) was carried out for five varieties *viz.*, Arka variety, IT- variety, TVX-944 variety, KM-5 and Gomati. Among these Arka variety, IT-35956-1 and TVX-944 were found to be resistance to *M. incognita.* Chlorophyll 'b' content in these inoculated resistance varieties was found to be reduced from 1.12 mg/g to 0.85 mg/g, 1.25 mg/g to 1.13 mg/g, and 1.32 mg/g to 1.21 mg/g respectively whereas KM-5 and Gomati showed susceptibility with values of 0.30 mg/g to 0.08 mg/g and 0.09 mg/g to 0.35 mg/g, respectively.

Among the susceptible varieties Gomati showed difference between inoculated and uninoculated cowpea varieties which was found to be noticed highest reduction per cent of chlorophyll "b" content in Gomati (74.29%) followed by KM-5 (73.33%). Among the resistant varieties there is decreased in chl b content of (24.11%) in Arka variety, (9.60%) in IT-35956-1 and (8.33%) in TVX-944 variety, respectively in comparison to uninoculated crop.

Among the susceptible varieties Gomati showed difference between inoculated and uninoculated cowpea varieties which was found to be noticed highest reduction per cent of chlorophyll "b" content in Gomati (74.29%) followed by KM-5 (73.33%). Among the resistant varieties there is decreased in chlor b content of (24.11%) in Arka variety, (9.60%) in IT-3596-1 and (8.33%) in TVX-944 variety, respectively in comparison to uninoculated crop is due to chlorophyll "b" accessory pigment acts indirectly in photosynthesis by transferring the light it absorbs to chlorophyll "a". Abiotic and biotic stresses damaged the plant leaf tissues, which was rapidly degrade the chloroplast and decreased the phosphorus, potassium, nitrogen, magnesium levels. After infection the photosynthetic efficiency and total chlorophyll content were reduced. Similar findings were obtained by [11, 3, 1, 12].

Table 2 Change in chlorophyll 'b' content (mg/g) of resistant and susceptible cowpea varieties due to infection of root-knot nematode,*Meloidogyne incognita*

SI NO.	Varieties	Chlorophyll content (mg/g) in leaves			
		Leaves			% increase(+)/Decrease(-)
		Inoculated	Uninoculated	mean	over uninoculated
1	Arka variety(R)	0.85	1.12	0.99	-24.11
2	IT-3596-1(R)	1.13	1.25	1.19	-9.60
3	TVX-944(R)	1.21	1.32	1.27	-8.33
4	KM-5(S)	0.08	0.3	0.19	-73.33
5	Gomati(S)	0.09	0.35	0.22	-74.29
	SE(M)±	0.09	0.14		
	CD(0.05)	0.25	0.43		
Resistant(R), Susceptible(S)					

Total Chlorophyll

The present study (**Table 3**) was carried out for five varieties *viz.*, Arka variety, IT-35956-1, TVX-944 variety, KM-5 and Gomati. Among these Arka variety, IT-35956-1 and TVX-944 variety were found to be resistance to *M. incognita*. Total Chlorophyll content in these inoculated resistance varieties was found to be reduced from 1.57 mg/g to 1.43 mg/g, 1.53 mg/g to 1.39 mg/g, and 1.77 mg/g to 1.58 mg/g respectively whereas KM-5 and Gomati showed susceptibility with values of 0.64 mg/g to 0.06 mg/g and 0.76 mg/g to 0.05 mg/g, respectively.

Among the susceptible varieties Gomati showed difference between inoculated and uninoculated cowpea varieties which was found to be noticed highest reduction per cent of total Chlorophyll content in Gomati (93.42%) followed

by KM-5 (90.63%). Among the resistant varieties there is decreased in total Chlorophyll content of (10.73%) in TVX-944 variety, (9.15%) in IT-35956-1 and (8.92%) in Arka variety, respectively in comparison to uninoculated crop.

Among the susceptible varieties Gomati showed difference between inoculated and uninoculated cowpea varieties which was found to be noticed highest reduction per cent of total Chlorophyll content in Gomati (93.42%) followed by KM-5 (90.63%). Among the resistant varieties there is decreased in total Chlorophyll content of (10.73%) in TVX-944 variety, IT-3596-1 (9.15%) and (8.92%) in Arka variety, respectively in comparison to uninoculated crop. due to chlorophyll "a" is identified as necessary pigment which converts light energy into chemical energy chlorophyll "b" accessory pigment acts indirectly in photosynthesis by transferring the light it absorbs to chlorophyll "a" which helps to absorb the light. Chlorophyll molecule has mg^{2+} at center which makes it ionic and hydrophilic and the ring hydrophobic in nature. Similar findings were obtained by [12, 1].

Table 3 Change in Total chlorophyll content (mg/g) of resistant and susceptible cowpea varieties due to infection of root-knot nematode,*Meloidogyne incognita*

SI NO.	Varieties	Chlorophyll content (mg/g) in leaves			
		Leaves			% increase(+)/Decrease(-) over uninoculated
		Inoculated	Uninoculated	mean	
1	Arka variety(R)	1.43	1.57	1.50	-8.92
2	IT-3596-1(R)	1.39	1.53	1.46	-9.15
3	TVX-944(R)	1.58	1.77	1.68	-10.73
4	KM-5(S)	0.06	0.64	0.35	-90.63
5	Gomati(S)	0.05	0.76	0.41	-93.42
	SE(M)±	0.08	0.10		
	CD(0.05)	0.23	0.27		

Resistant(R), Susceptible(S)

Conclusion

In conclusion, the present study suggested that clearly indicated that M*eloidogyne incognita* played key role in altering the normal physiology of the tested host plant. Further, Basic studies relating to physiology mechanism of resistance in cowpea to the Root knot nematode made elucidate the physiology basis of resistance to host to the nematode observation were made in the changes in physiology parameters.

- Chlorophyll a, b and total chlorophyll content decreases in leaves of infected plants as compared to control.

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