The Pharma Innovation Journal 2022; SP-11(10): 2276-2279

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(10): 2276-2279

www.thepharmajournal.com
Received: 12-07-2022
Accepted: 18-09-2022

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Interaction effects of root knot nematode and rhizobium on chlorophyll content in different field pea cultivars

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Abstract
The pot culture experiment was conducted during Rabi season in the year 2021- 2022 at Green polyhouse condition, Department of Nematology, OUAT, Bhubaneswar, Odisha, in order to study Interaction Effects of Root Knot Nematode and Rhizobium on Chlorophyll Content in Different Field Pea Cultivars. The experiment was laid out in Complete randomized design with 7 treatments i.e T_1= Nematode (1000 J_2/pot), T_2= Rhizobium T_3= Nematode + Rhizobium (same time), T_4= Nematode + Rhizobium (after 10 days of nematode inoculation), T_5= Rhizobium + Nematode (after 10 days of rhizobium inoculation), T_6= Carbofuran @ 2 kg ai/ha (0.15g/pot) a nd T_7= Untreated check. Above all combination of treatment were applied to the three germplasms named as IPFD-5-19 (AMAN), IPFD-10-12 and IPFD-1-10 (PRAKASH), which were showing Resistant (R), Moderately resistant (MR) and Susceptible (S) reaction against root knot nematode respectively. Decrease of chlorophyll content was maximum in highly susceptible variety IPFD-1-10 (chl a- 45.44%, chl b- 52.06%, Total chl- 63.66%) and that of minimum in resistant variety IPF-5-19 (chl a- 23.48%, chl b- 37.23%, total chl- 41.94%) in nematode inoculated plant over check. There is highest increase in chlorophyll content in rhizobium inoculated plant (T_2) and then application of carbofuran (T_6) over the check (T_7).

Keywords: Root knot nematode, *M incognita*, field pea, chlorophyll (a, b and total)

Introduction
Pea (*Pisum sativum* L.) was used by Mendel to lay the foundation of modern genetics (Yang *et al*., 2015) [1]. It is one of the major food legumes that can grow in different regions and rich in proteins, vitamins, minerals, carbohydrates, and seed oil (Rungruangmaitree *et al*., 2017) [2]. Pea is predominantly a self-pollinated crop with limited variation in the number of flowers per node (Esawi *et al*., 2018) [3]. Most garden pea germplasm/varieties lines have either one or two flowers per node (Devi *et al*., 2018) [4]. Chlorophyll content is the most important constituent of the plants as it manufactures the food, which is necessary for the growth and development of the plant. It is directly correlated with the yield of the crops. Root-knot nematodes are known to reduce the chlorophyll content of plants by disrupting its nutrient uptake and partitioning of the photosynthates. But various rhizosphere organism are present in the soil, which interacted with the root knot nematode. In the rhizosphere, Rhizobium fixes atmospheric nitrogen and produces toxic metabolites inhibitory to many plant pathogens. The dual symbiosis (AM Fungi and Rhizobium) exerts a synergistic effect on plants. Besides increasing plant growth, this symbiosis also imparts resistance against endo parasitic nematodes (Akhtar and Siddiqui, 2008) [5].

Materials and Methods
Here three field pea germplasms namely IPFD-5-19 (AMAN), IPFD-10-12 and IPFD-1-10 (PRAKASH), which are resistant, moderately resistant and susceptible respectively against the root knot nematode were taken out for studying the change in chlorophyll (a, b, total) content.

Preparation of soil and pots
Soil was mixed in a ratio of 2:1:1 with sand and FYM, which was packed in a gunny bag and fumigants incorporated in the soil to kill all the nematode if present and microorganisms like bacteria, fungi etc. This process is important for get a sterilized soil for the future experiment purposes.

Sowing of seeds

Field pea germplasms seeds are sowed in the pot. 4-5 number of field pea seeds are sowed in the plot. After the germination only one or two healthy seedling allowed to grow for further purposes.

Inoculation of Nematodes and Rhizobium

Previously cultured 2nd stage juvenile (J_2) of Root knot nematode (*Meloidogyne incognita*) and *Leguminosarum* strain of Rhizobium inoculated in the pot after 15 days of the sowing of the seeds in various combination. After the 45 days of inoculation of the nematodes and rhizobium, readings for the chlorophyll content of the leaves (% fresh weight basis) were taken out.

One gram of 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 (Lichtenthaler and Welburn, 1983) [6].

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

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

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

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 gram of corresponding amount of fresh leaves used in the extraction of chlorophyll.

Treatment Details

1. T_1= Nematode (1000 J_2/pot)
2. T_2= Rhizobium
3. T_3= Nematode + Rhizobium (same time)
4. T_4= Nematode + Rhizobium (after 10 days of nematode inoculation)
5. T_5= Rhizobium + Nematode (after 10 days of rhizobium inoculation)
6. T_6= Carbofuran @ 2kg ai/ha (0.15g/pot)
7. T_7= Untreated check

Results

Chlorophyll ‘a’, ‘b’ and total (mg/g fresh weight) present in leaves were described in Table.1, 2, 3. Chlorophyll ‘a’ was decreased in T1 recorded 0.69, 0.72, 0.76 mg/g fresh leaf in IPFD-5-19(R), IPFD-10-12(MR) and IPFD-1-10(S) respectively over T7 (Check). In T2 and T6 where only Rhizobium and carbofuran was inoculated there was increased in Chlorophyll ‘a’ in all varieties over check. The highest percent increase was 26.24% in IPFD-5-19(R), 36.77% in IPFD-10-12 and 52.59% in IPFD-1-10 over check. Compared with T1 the reduction was seen minimum in T4, T3 and T5 over check where combination of both nematode and rhizobium were inoculated in different time.

In case of chlorophyll ‘b’ there was reduction in infected plant compared with healthy check. Maximum reduction was seen in T1 of IPFD-1-10 i.e. from 1.35to 0.90 mg/g having 52.09 percent over check and minimum reduction in IPFD-5-19 variety from 1.10 to 0.74 mg/g having percent decrease of 37.23% over check (T7). Less reduction of chlorophyll ‘b’ was seen in T5 where rhizobium was applied before nematode inoculation having 6.81% in IPFD-5-19, 17.20% in IPFD-10-12 and 28.23% in IPFD-1-10 over check (T7).

Total chlorophyll content decreased in all treatments except T2 and T6 in all varieties over check (T7). There was highest reduction in T1 followed by T4, T3 and T5 in descending order and the lowest being T5 having 9.82%, 20.95% and 21.03% in in IPFD-5-19(R), IPFD-10-12(MR)and IPFD-1-10(S) respectively over check. It was noticed that treatment where Rhizobium was applied alone there was significant increase in total chlorophyll content in all varieties.

Table 1: Effect of *M. incognita* and *Rhizobium* either alone or in combination on chlorophyll ‘a’, ‘b’ & ‘total’ content (mg/g leaf) of resistant germplasm of field pea Leaf (Var. IPF-5-19)

Treatments	Chl a	% Change	Chl b	% Change	Total Chl	% Change
T1 (N)	0.69	-23.48	0.74	-37.23	1.20	-41.94
T2 (RHI)	1.14	26.24	1.55	31.91	2.74	32.85
T3 (N+R)	0.77	-15.19	0.94	-19.79	1.66	-19.52
T4 (N→R)	0.73	-19.06	0.84	-28.94	1.46	-29.21
T5 (R→N)	0.81	-10.77	1.10	-6.81	1.86	-9.82
T6 (C)	0.98	8.29	1.29	10.00	2.31	11.76
T7 (control)	0.91		1.18		2.06	
SE(m) ±	0.03		0.03		0.09	
CD	0.09		0.08		0.27	

Table 2: Effect of *M. incognita* and *Rhizobium* either alone or in combination on chlorophyll 'a', 'b' & 'total' content (mg/g leaf) of moderately resistant germplasm of field pea Leaf (Var.IPFD-10-12)

Treatments	Chl a	% Change	Chl b	% Change	Total Chl	% Change
T1 (N)	0.72	-38.49	0.75	-40.20	1.32	-44.34
T2 (RHI)	1.59	36.77	1.74	39.20	3.55	50.05
T3 (N+R)	0.96	-17.42	0.99	-21.20	1.81	-23.60
T4 (N→R)	0.91	-22.15	0.92	-26.80	1.54	-35.03
T5 (R→N)	1.03	-11.83	1.04	-17.20	1.87	-20.95
T6 (C)	1.28	9.68	1.39	11.00	2.66	12.70
T7 (control)	1.16		1.25		2.36	
SE(m) ±	0.05		0.03		0.08	
CD	0.15		0.09		0.24	

Table 3: Effect of *M. incognita* and *Rhizobium* either alone or in combination on chlorophyll 'a', 'b' & 'total' content (mg/g leaf) of susceptible germplasm of field pea Leaf (Var.IPFD-1-10)

Treatments	Chl a	% Change	Chl b	% Change	Total Chl	% Change
T1 (N)	0.76	-45.44	0.90	-52.06	1.19	-63.33
T2 (RHI)	2.13	52.59	3.01	60.45	5.31	63.71
T3 (N+R)	1.09	-22.18	1.28	-31.96	2.51	-22.73
T4 (N→R)	0.95	-32.38	1.10	-41.28	2.35	-27.73
T5 (R→N)	1.22	-12.88	1.35	-28.23	2.56	-21.03
T6 (C)	1.58	13.06	2.18	15.98	3.75	15.41
T7 (control)	1.40		1.88		3.25	
SE(m) ±	0.04		0.06		0.08	
CD	0.12		0.18		0.24	

(N indicates Nematode, RHI indicates Rhizobium, N+R indicates both nematode and rhizobium inoculated at same time, N→R indicates rhizobium inoculated after 10 days of nematode inoculation, R→N indicates nematode inoculated after 10 days of inoculation of rhizobium and C indicates Carbofuran)

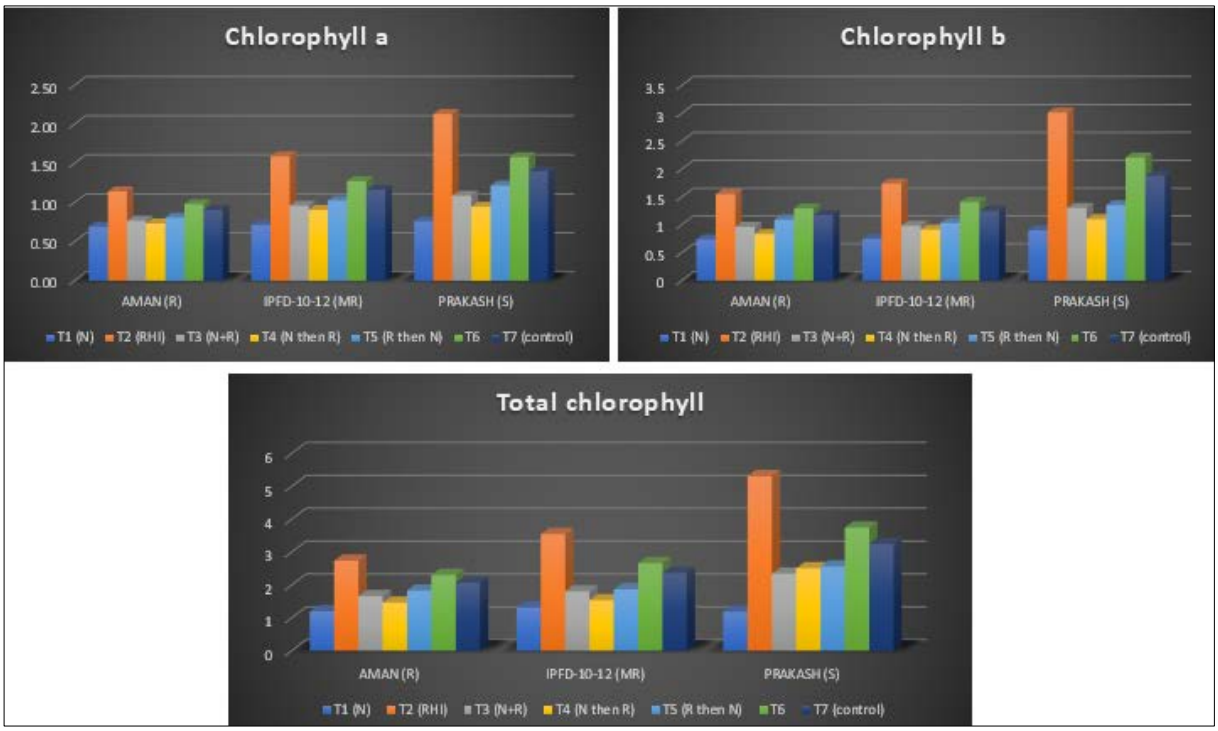

Fig 1: Above all three chat shows variation in the Chlorophyll a, b & total content of three germplasm namely AMAN, IPFD-10-12 & PRAKASH in different combination of treatment

Discussion

In all three germplasms Rhizobium treated plants shows maximum increase over the all other treatment combination. Rhizosphere microorganisms provide a front line defense for root pathogen attack. These micro-organisms utilize compound and materials released from the crop roots and roots in turn provide nutrition to the microorganisms (Siddiqui and Akhtar, 2009) [7]. M. incognita reduced the chlorophyll and nutrient contents in all the plants as compared to the chickpea plants in its absence reported by Rizvi *et al.* [8] which is agreed with in this experiment. In all three germplasm only root knot nematode infected plants showing measure decreasing trend as compared with the control. Prior application of rhizobium before root knot nematode inoculation shows the significance difference than the prior inoculation of root knot nematode before rhizobium to the all

plants, which shows similar result as reported by the Nayak *et al*., 2020 [9].

Conclusion

In conclusion our results indicate spectacular changes in chlorophyll content occur in susceptible and resistant cultivars of field pea after inoculating rhizobium and root-knot nematode (*Meloidogyne incognita*) either alone or in combination. Further we intended to screen some physiological indices which can be used for further study to develop nematode resistant field pea cultivars

Acknowledgement

The authors are thankful to IIPR (Indian Institute Of Pulse Research), Kanpur Uttar Pradesh for providing seed and Department of Nematology and Plant physiology, College of Agriculture, OUAT, Bhubaneswar, Odisha for providing space for research work.

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