International Journal of Plant & Soil Science

34(23): 490-495, 2022; Article no.IJPSS.92543
ISSN: 2320-7035

Variation in Root Nutrient Content in Different Field Pea Germplasms Infected with Root Knot Nematode

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2022/v34i2331614

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/92543

Original Research Article

Received 02 August 2022
Accepted 05 October 2022
Published 10 October 2022

ABSTRACT

This study was conducted in the Department of Nematology, College of Agriculture, OUAT. The experiment was designed using completely randomized completely randomized design (CRD) with different combinations of root knot nematode (*Meloidogyne incognita*) and leguminosarum strain of rhizobium in the three field pea germplasms, which are resistant, moderately resistant and susceptible against the root knot nematode infection. Different combination of interaction between root knot nematode and rhizobium affect the nutrient content likely nitrogen, phosphorus and potassium uptake of the roots of field pea germplasms. Different combination of interaction between root knot nematode and rhizobium shows the decreased trend of nutrient uptake than that of only rhizobium treated plants

Keywords: Root knot nematode (Meloidogyne incognita); rhizobium; nutrient; field pea.

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1. INTRODUCTION

Field pea is one of the oldest domestic pulse crops, appearing in the Mediterranean between 7000 and 6000 BC and persisting in current agriculture [1]. Field pea planted during both rabi and summer, but in Odisha condition it planted during the month of November and December. Pulses are able to break disease and weed cycles associated with cereals by replenishing nitrogen (N) in the soil through their ability to fix N from the atmosphere through their nodules and symbioses with rhizobia. As N is another of the most limiting nutrients for cereal and crop production, this legume-mediated increase in nitrogen use efficiency offers a sustainable and cost-effective alternative to high input fertilizer regiments. Pulses also foster other beneficial properties for soil health, such as improving biodiversity, soil organic carbon (SOC) levels, and soil water retention, while reducing greenhouse gas emissions (GHG) [2,3,4]. In 2017, a total of 8,141,031 hectares of field pea were harvested globally, with the top producers consisting of Canada, Russia, China, India, and the United States [5]. Cereals have less protein than field pea and pulse crops as well as inadequate levels of micronutrients, which contributing to hidden hunger [6]. Pulses are also good sources of prebiotic carbohydrates (essential for gut health), fiber, minerals, vitamins, carotenoids, and polyphenols, allowing them to address health problems such as malnutrition, prenatal care, cardiovascular disease, diabetes, cancer, obesity, and gastrointestinal (GI)-related issues that plague both developing and developed nations [7,2]. Due to the nematode infection around 21% yield loss noticed in worldwide. The root knot nematodes while attacking the roots of legume crops also affect the development of rhizobial nodules and vice-versa [8]. Root knot nematode is semi-endo parasitic nematode. 2nd stage juvenile of this nematode attack the plants roots. Stylet of this nematode piercing the plant root and malformed the root system by forming giant cell which is the result of hyperplasia. As this crop planted during the rabi season, there were residual moisture present in the soil, which makes the nematode favorable for thrive. Root knot nematode can not reproduce in high temperature regime. So the relatively cool temperature during the planting month of the field pea favors for the nematode infection.

Here the objective of the study to know how the nematode infection lower the nutrient uptake.

2. MATERIALS AND METHODS

Here three field pea germplasms namely Prakash, IPFD-10-12 and Aman, which are susceptible, moderately resistant and resistant respectively against the root knot nematode were taken out for studying the change in nutrient content of the root.

2.1 Preparation of Soil and Pots

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

2.2 Sowing of Seeds

Three to four field pea germplass seeds are in the pot. After the germination only two healthy seedling is allowed to grow for further experiment purposes.

2.3 Inoculation of Nematodes and *Rhizobium*

After 15 days of sowing the seeds in various combinations, previously cultured 2nd stage juvenile (J2) Root Knot Nematode (*Meloidogyne incognita)* and Leguminosarum strain of *Rhizobium* were inoculated in the pot. After the 45 days of inoculation of the nematodes and rhizobium, readings for the nutrient content on the root (% dry weight basis) were calculated.

2.4 Treatment Details

Following treatments were applied to the each pot.

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

2.5 Estimation of Nitrogen of Roots

Nitrogen content of the roots were estimated by following the procedure of Mahadevan and Sridhar [9]. Two hundred mg of powdered plant parts were taken in 100 ml micro Kjeldahl digestion flasks. About 200 mg of digestion mixture (K_2SO_4: $CuSO_4$ = 5:1) and 4 ml of concentrated H_2SO_4 were added. These flasks were kept as such for about one hour and then heated slowly till frothing occurred. To check the frothing, two crystals of sodium thio-sulphate were added to each digestion flask. Thereafter, digestion was continued until the contents of the flask became completely clear blue syrupy liquid without any bubbling. The flask was cooled and content was diluted to 25 ml with distilled water. Then 10 ml of diluted sample extract was transferred into micro Kjeldahl distillation unit. Thereafter, 10 ml of 40 % NaOH was added and distillation was continued for 10 minutes. During distillation period, liberated ammonia was absorbed by 150 ml conical flask containing 2 drops of mixed indicator. After completion of distillation, distillate was titrated against 0.05N H_2SO_4.

2.6 Calculation

$$\text{Percent } N_2 \text{ in sample} = \frac{(\text{Sample titer - blank titer}) \times N_2 \text{ of } H_2SO_4 \times 14 \times 100 \times 2.5}{\text{Sample weight (g)} \times 1000}$$

2.7 Estimation of Phosphorus

Phosphorus content in root samples was estimated by adopting the procedure of Jackson [10].

2.8 Chemical Reagents

8. Molybdate – Vanadate solution

   a) Dissolve 6.250 g ammonium molybdate in 125 ml of distilled water.
   b) Dissolve 313 mg ammonium Vanadate in 125 ml of 1(N) HNO_3

Then mix the reagents (a) and (b) in a 250 ml volumetric flask
The resulting solution is called molybdate – vanadate solution.

9. 2(N) HNO_3: Dilute the 60 ml concentrated HNO_3 to 480 ml with distilled water.
10. The standard phosphorus solution (25 ppm): Dissolve 55 mg monobasic potassium phosphate (KH_2PO_4) in distilled water and dilute to 500 ml.

2.9 Sample Analysis

Standards of 0, 2.5, 5.0, 7.5 and 10.0 ml of 25 ppm phosphorus solution and 2 ml of digested sample extracts were taken in 25 ml volumetric flasks. Five ml of 2N HNO_3 solution was added to each flask. Then required amount of distilled water was added to each flask to make the final volume 15 ml. Thereafter, 2.5 ml molybdate - vanadate solution was added. Final volume was made up to 25 ml with distilled water and flasks were shaken well. Absorbance was measured by a spectrophotometer at 420 nm after 20 minutes of shaking. The phosphorus content of root samples was calculated in percentage by using the standard curve.

2.10 Estimation of Potassium

1 ml digested sample extract of root were taken in 25 ml volumetric flasks and the volume was adjusted to 25 ml with distilled water. Similarly 1, 2, 3, 4 and 5 ppm standard K solution were taken in 100 ml volumetric flasks with water. The readings for standards and samples were taken in a digital flame photometer. As per the standard curve, the concentration of potassium present in extracting solution was calculated. Then the percentages of potassium present in root samples were calculated.

3. RESULTS

3.1 Change in Nitrogen Content (Dry Weight %) in Roots (Table 1)

The percentage of nitrogen content in all three varieties increases over the control but maximum are recorded in the T_2 treatment followed by T_5, T_3 and T_4 treatment(Table1).The application of T5 produced the best increase in nitrogen content, followed by T4, T2, T3 and finally T1 treatment.

Only nematode infected plants (T_1)are also showing the increased in nitrogen content over the control. But when compared among the

varieties, susceptible variety shows the maximum root nitrogen content than that of resistant and moderately resistant varieties.

3.2 Change in Phosphorus Content (Dry Weight %) in Roots (Table 2)

The percentage of phosphorus content in all three varieties increases over the control but maximum increases recorded in the T2 treatment followed by T5, T3 and T4 treatment. Only nematode infected plants (T_1) are also showing the increased in phosphorus content over the control. The application of T5 produced the best increase in phosphorus content, followed by T4, T2, T3 and finally T1 treatment. The percentage of phosphorus content in all three varieties was diffracted by the application various combination of treatments. But when compared among the varieties, susceptible variety shows the maximum root phosphorus content than that of resistant and moderately resistant varieties.

3.3 Change in Potassium Content (Dry Weight %) in Roots (Table 3)

In all the three varieties percentage potassium content increases over the control but maximum increases recorded in the T_2 treatment followed by T_5, T_3 and T_4. Only nematode infected plants (T_1) are also showing the increased in potassium content over the control. But when compared among the varieties, susceptible variety shows the maximum root potassium content than that of resistant and moderately resistant varieties. But change in root potassium content in T_1 and T_6 over the control relatively less than the other treatment.

Table 1. Nitrogen content in the roots(dry weight %) of different germplasms infected with nematode and *rhizobium*

Treatments	AMAN (R)		IPFD-10-12 (MR)		PRAKASH (S)	
	Root	Change over control(%)	Root	Change over control(%)	Root	Change over control(%)
T1 (N)	**0.63**	26.63	**0.72**	31.05	**0.77**	37.22
T2 (RHI)	**0.76**	53.27	**0.85**	54.79	**0.87**	55.61
T3 (N+R)	**0.67**	34.17	**0.75**	36.07	**0.78**	39.46
T4 (N then R)	**0.69**	39.20	**0.77**	41.10	**0.79**	42.15
T5 (R then N)	**0.71**	43.22	**0.80**	45.21	**0.81**	45.74
T6	**0.58**	16.58	**0.64**	16.89	**0.65**	17.04
T7 (control)	**0.50**		**0.55**		**0.56**	
SE(m)±	0.01		0.02		0.01	
CD(0.05)	0.04		0.05		0.04	

N indicates Nematode, RHI indicates Rhizobium, N+R indicates both nematode and rhizobium inoculated at same time, N then R indicates rhizobium inoculated after 10 days of nematode inoculation, R then N indicates nematode inoculated after 10 days of inoculation of rhizobium

Table 2. Phosphorus content in the roots(dry weight %) of different germplasms infected with nematode and *rhizobium*

Treatments	AMAN (R)		IPFD-10-12 (MR)		PRAKASH (S)	
	Root	Change over control(%)	Root	Change over control(%)	Root	Change over control(%)
T1 (N)	**0.31**	14.81	**0.33**	16.67	**0.36**	18.85
T2 (RHI)	**0.41**	50.93	**0.44**	53.51	**0.48**	55.74
T3 (N+R)	**0.33**	21.30	**0.36**	25.44	**0.39**	26.23
T4 (N then R)	**0.31**	15.74	**0.34**	19.30	**0.37**	19.67
T5 (R then N)	**0.33**	22.22	**0.37**	28.07	**0.40**	30.33
T6	**0.30**	11.11	**0.32**	12.28	**0.35**	15.57
T7	**0.27**		**0.29**		**0.31**	

Treatments	AMAN (R)		IPFD-10-12 (MR)		PRAKASH (S)	
	Root	Change over control(%)	Root	Change over control(%)	Root	Change over control(%)
(control)						
SE(m)±	0.01		0.01		0.01	
CD(0.05)	0.04		0.04		0.03	

N indicates Nematode, RHI indicates Rhizobium, N+R indicates both nematode and rhizobium inoculated at same time, N then R indicates rhizobium inoculated after 10 days of nematode inoculation, R then N indicates nematode inoculated after 10 days of inoculation of rhizobium

Table 3. Potassium content in the roots(dry weight %) of different germplasms infected with nematode and *rhizobium*

Treatments	AMAN (R)		IPFD-10-12 (MR)		PRAKASH (S)	
	Root	Change over control(%)	Root	Change over control(%)	Root	Change over control(%)
T1 (N)	**1.42**	18.05	**2.04**	20.18	**2.14**	21.28
T2 (RHI)	**2.12**	75.93	**3.02**	78.06	**3.18**	80.57
T3 (N+R)	**1.91**	58.30	**2.74**	61.27	**2.89**	63.83
T4 (N then R)	**1.79**	48.76	**2.57**	51.10	**2.70**	53.19
T5 (R then N)	**2.01**	66.60	**2.86**	68.63	**3.01**	70.78
T6	**1.45**	19.92	**2.06**	21.21	**2.17**	22.98
T7 (control)	**1.21**		**1.70**		**1.76**	
SE(m)±	0.03		0.08		0.05	
CD(0.05)	0.08		0.24		0.15	

N indicates Nematode, RHI indicates Rhizobium, N+R indicates both nematode and rhizobium inoculated at same time, N then R indicates rhizobium inoculated after 10 days of nematode inoculation, R then N indicates nematode inoculated after 10 days of inoculation of rhizobium

4. DISCUSSION

Maximum nutrient uptake shown in the *rhizobium* treated plants in all three varieties due to the fixation of free nitrogen [11]. As rhizobium is a symbiotic nitrogen fixing bacteria, it increases the plant nutrient uptake by fixing thee free atmospheric nitrogen which ultimately used by the plants. In all three varieties of field pea, nitrogen, phosphorus and potassium content in root decreases in all the treatment except treatment where only rhizobium treatments were given. Because interaction between the nematode and rhizobium in the root system of the plant, interaction affects the nutrient uptake capacity of the plants. Earlier studies reveal that root knot nematode infection reduce the nodule size, nodule number and also transform the functional nodules into the non-functional nodules [12,13]. Due to the interaction of the root knot nematode with *rhizobium*, nodules formation affected in the plant system which reduce the nutrient uptake of the plants which is same as of our experiment. Reduced nodulation due to nematode infection may be attributed to secretion of hydrolytic and oxidative enzymes [14], competitive phenomenon between rhizobia and nematodes [15] and interference of juveniles with the establishment of rhizobia [16]. In all the treatment where root knot nematode inoculated there was decrease in nutrient content as compared with the treatment where only *rhizobium* were inoculated.

5. CONCLUSION

Rhizobium inoculated plants shows maximum increases of N, P & K over the control in all cultivars. But susceptible cultivars show the maximum deposition of N, P & K in the root zone because of malformed root, which is done by the nematode infection. Due to the nematode infection roots are modified and giant cells are formed that is the reason of deposition of N, P & K in the root zone. As it is occurred maximum in the susceptible varieties because of the heavy infection of nematodes. But if we applied *rhizobium* with nematode, nutrient status increases over all the treatment except only rhizobium inoculated plants. So prior to the planting of the field pea in the field if plant seeds are treated with rhizobial culture or soil

application of rhizobium in the field carried out then the nematode infestation decreases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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