**Variation in root nutrient content in different field pea germplasms infected with the Root knot Nematode**


**Abstract**

A This study was conducted in the DeptDepartment of nematology, College of Agriculture, OUAT. The experiment laid out using Ccompletely Rrandomized Ddesign (CRD) with different combination 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 affects 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 decrease trends of nutrient uptake than that of only rhizobium treated plants.



Keywords: Root knot nematode (*Meloidogyne incognita*), Rhizobium, Nutrient, Field pea


**Introduction**

Field pea is one of the oldest domesticated pulse crops, appearing in the Mediterranean between 7000 and 6000 BC and persisting in current agriculture [1]. Pulses are able to break disease and weed cycles associated with cereals, while 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 increased biodiversity, soil organic carbon (SOC) levels, and soil water retention, while decreasing greenhouse gas emissions (GHG) [2][3][4]. Field pea has the most positive effect on SOC by improving humus levels and supplying organic C as a result of bacterial nitrogen fixation [3]. 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]. As cereals have less protein than field pea and pulse crops as well as inadequate levels of micronutrients, 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].



**Materials and methods**

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

**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 id important for get a sterilized soil for the future experiment purposes.

**Sowing of seeds**

Field pea germplasms seeds are sowed in the pot. Generally 3-4 number of field pea seeds are sowed in the plot. After the germination only one healthy seedlings 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 45days of inoculation of the nematodes and rhizobium, readings for the nutrient content on the root (% dry weight basis) were taken out.

**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

**Estimation of nitrogen of roots**

Nitrogen content of roots were estimated by following the procedure of Mahadevan and Sridhar[8]. 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 $H_2SO_4$.

**Calculation**

Percent $N_2$ in sample **=**

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

**Estimation of phosphorus**

Phosphorus present in plant samples was estimated by adopting the procedure of Jackson [9].

**Chemical reagents**

1. 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.
2. 2(N) $HNO_3$: Dilute the 60 ml concentrated $HNO_3$ to 480 ml with distilled water.
3. The standard phosphorus solution (25 ppm): Dissolve 55 mg monobasic potassium phosphate ($KH_2PO_4$) in distilled water and dilute to 500 ml.

**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 plant samples was calculated in percentage by using the standard curve.

**Estimation of potassium**

One 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 (i.e. 0.1907 g KCL/~~lit~~liter) 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 ppm of potassium present in extracting solution was calculated. Then the percentages of potassium present in shoot samples were calculated.

**Results**

**Change in Nitrogen content (~~%~~ dry weight %) in roots (~~Table~~ Table 1):**

In all the three varieties percentage nitrogen content increases over the control but maximum increases recorded in the $T_2$ treatment followed by $T_5$, $T_3$ and $T_4$ (~~Table~~ Table 1). 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.

**Change in Phosphorus content (~~%~~ dry weight %) in roots (~~Table~~ Table 2):**

In all the three varieties percentage phosphorus 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 phosphorus content over the control. But when compared among the varieties, susceptible variety shows the maximum root phosphorus content than that of resistant and moderately resistant varieties.

**Change in Potassium content (~~%~~ dry weight %) in roots (~~Table~~ 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.

**Discussion:**

Maximum nutrient uptake shown in the rhizobium treated plants in all three varieties due to the fixation of free nitrogen[10].The root-knot nematodes while attacking the roots of legume crops also affect the development of rhizobial nodules and vice-versa [11]. 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.

~~Table~~ Table 1: Shows the results of change in the Nitrogen content in the roots(~~%~~ dry weight %)

| Treatments | PRAKASH (R) | | IPFD-10-12 (MR) | | AMAN (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~~ Table 2: Shows the results of change in the Phosphorus content in the roots(~~%~~ dry weight %)**

| Treatments | PRAKASH (R) | | IPFD-10-12 (MR) | | AMAN (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 (control)** | **0.27** | | **0.29** | | **0.31** | |
| **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~~ Table 3: Shows the results of change in the Potassium content in the roots (~~%~~ dry weight %)

| Treatments | PRAKASH (R) | | IPFD-10-12 (MR) | | AMAN (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.

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