World Applied Sciences Journal 10 (2): 242-248, 2010
ISSN 1818-4952

Determination of Nitrogen and Phosphorus Uptake and Fixed N Measurement in Pea Genotypes in Southeast Ethiopia

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Abstract: Nitrogen and phosphorus uptake and fixed N in pea genotypes due to inoculation of rhizobial strains were assessed in soils of Sinana, situated within latitude 07°07' N of the equator and longitude 40°10' E of Greenwich Meridian at altitude 2400 m above sea level. Two strains (EAL 300 and EAL 302) of *Rhizobium leguminosarum* and their combination were evaluated for their interaction with three varieties of pea grown under field conditions. The residual soil nitrogen as a result of inoculation of *rhizobial* strain EAL 302 increased by 13% and 31% compared to uninoculated and EAL 300, respectively. Varietal response revealed that Wayitu increased the post harvest total soil nitrogen by 20% than the local variety. The increase in P uptake, residual nitrogen and fixed nitrogen were significantly high due to inoculation of pea varieties with *rhizobial* strains. The Wayitu variety and *Rhizobium* strain EAL 302 resulted in an increment of 19% and 46% of the fixed N than local and EAL 300 from nitrogen fixation in the study area respectively. Thus, on the bases of competitive ability inocula of strain EAL 302 on pea variety Wayitu performed better with respect to response of nitrogen and phosphorus uptake and nitrogen fixation in Sinana area.

Key words: Interaction · Nodulation · *Rhizobium leguminosarum* · N-fixation · Inoculation

INTRODUCTION

Biological nitrogen fixation (BNF) is an efficient source of nitrogen [1] and [2]. The total annual terrestrial inputs of N from BNF according to Burns and Hardy [3] and Paul [4] range from 139 million to 175 million tones of N, with symbiotic associations growing in arable land accounting for 25 to 30% (35 million to 44 million tons of N).

The contribution of any N_2-fixing plant to the sustainability of agricultural systems depends on how much of the fixed N is harvested and removed from the soil. For grain legumes the amount of N contributed is the amount of N_2-fixed less the amount of N harvested in the seed [5].

A tremendous potential for contribution of fixed N to soil ecosystems exists among the legumes [2] and [6]. Sprent [7] reveals that there are approximately 700 genera and about 13,000 species of legumes, only a portion (about 20%) have been examined for nodulation and shown to have the ability to fix- N_2.

Therefore, the objectives of this study was to assess the effect of inoculation of different strains of *R. leguminosarum* and their combination on N and P uptake and amount of fixed N in soils of Sinana.

MATERIALS AND METHODS

The experiment was conducted on silty-loam soil at farmer's field under continuous cereal production systems near Sinana Agricultural Research Center, situated within latitude 07°07' N of the equator and longitude 40°10' E of Greenwich Meridian at altitude 2400 m above sea level, from August to December main 2005 cropping season. The area is typically characterized by bimodal rainfall having two distinct growing season *viz.,* (March-July) and (August–December). The annual mean annual rainfall is 50.69mm and the average annual maximum and minimum temperature of 2005 is 21 °C and 9.51°C, respectively.

Experimental Detail: Carrier peat based inoculants of *Rhizobium leguminosarum* strains EAL 300 and EAL 302, were obtained from the Soil Microbiology Laboratory of National Soil Research Center (NSRC), Addis Ababa, Ethiopia.

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Experimental Design and Treatments: The experimental design was randomized complete block design (RCBD) in a 4 × 3 factorial combination of inoculant strains (EAL 300, 302, uninoculated and combination of EAL 300 + EAL 302) and Pea genotypes (Local, Wayitu and Dadimos). The plot size was 3x 4m and spacing between plots and blocks were 1m and 1.5m, respectively. Ridges were made between each plot to reduce the movement of bacteria from plot to plot. For the determination of the amount of N-fixed the non-fixing reference crop (Barley) was used.

Triple super phosphate and urea was applied uniformly to the treatments at sowing in the seed row at the rate of 46 kg $P_2O_5ha^{-1}$ and 20 kg Nha^{-1} respectively.

Seed Inoculation: All the Seeds genotypes (Local, Wayitu and Dadimos) were surface sterilized by successive treatment with 0.1% $HgCl_2$ solution and absolute alcohol and later washed five times with sterilized water. The seeds were inoculated with cool and clean water at the rate of 7ml per kg of seeds using 1:1 slurry of peat-based inoculants of *Rhizobium leguminosarum* strains. All inoculants were applied prior to sowing under shade to maintain the viability of cells and seeds were air dried before sowing.

Sowing: Pea varieties were sown one seed per hole at a rate of 150 kg ha^{-1}on August 30, 2005 in ten rows of each plot at spacing of 30 cm apart and 10 cm within row. The non-inoculated plots were first sown in order to avoid contamination and then all treatments with the same *rhizobia* strain(s) were sown in this order.

Agronomic Practices: The first weeding was done manually with hoe? [T5]40 days after sowing and the second weeding one month later. Prior to flowering and at about pod forming stage of the crop, Karate insecticide *was* sprayed at the rate of 0.04 lit/ha to control aphids and ball worms, respectively.

Soil Sample Collection and Analysis: Composite surface soil samples (0-30 cm) was randomly collected from 10 sampling spots of the entire experimental site before sowing for the determination of physico-chemical properties of the soil. The collected samples were air dried at room temperature and ground to pass through a 2 mm sieve.

Soil bulk density was determined by core method and soil pH by potentiometric method 1:2.5 soil water ratio [8]. Cation exchange capacity was determined by 1M ammonium acetate method at pH 7 [9]. Organic carbon by dichromate oxidation method [10] and total nitrogen by the micro kjeldhal method [11], available P was analyzed as described by [12] and determined colorimetrically by the ascorbic acid-molybdate blue method [13]. Ca^{++} and Mg^{++} by Atomic Absorption Spectrophotometer, $Na^{+}$and K^{+} was determined using flame photometer. Soil particle size distribution was determined by hydrometer method [14].

Plant Sample Collection and Processing: At physiological maturity, two non-border plant rows were harvested and partitioned into grain and straw. The grain and straw sample materials was oven dried at 70°C to a constant weight and then ground for tissue analysis of grain and straw respectively.

Determination of N Uptake in Grain and Straw: Total N in grain and straw sub-samples was determined by kjeldahl procedure [15]. Nitrogen uptake in the grain and straw was determined quantitatively by multiplying nitrogen content of the grain and straw with that of grain and straw yield respectively. The total amount of nitrogen was calculated as the sum of grain N uptake and straw N uptake.

Determination of P Uptake in Grain and Straw: Phosphorus in grain and straw sub-samples was determined using meta vanadate method [16]. Samples were calcinated in the furnace for 24 hours at 450°C and the ash dissolved in 20% nitric acid (HNO_3) to liberate organic phosphorus. The phosphorus in solution was determined colorimetrically with molybdate and metavanadate for color development and read on spectrophotometer. The values of Phosphorus uptake in grain and straw was multiplied with the P content in grain and straw yield values, to obtained levels of P uptake respectively. Total P uptake was calculated as the summation of grain and straw uptake.

Measurement of Biological Nitrogen Fixation

N-Difference Method: Field estimation of N_2 fixation was determined by measuring the total amount of N in the legume crop and the nitrogen content in a non-fixing reference crop (barley). The nitrogen content remained in the soil after harvest from legume field and reference crop field was also analyzed for the determination of nitrogen fixed [17].

Beck *et al* [17] who stated the quantity (Q) of N derived from N_2 fixation symbiotically as:

$$Q = [N\ yield\ (leg) - N\ yield\ (ref)] + [N\ soil\ (leg) - N\ soil\ (ref)]$$

Statistical Analysis: Data were analyzed using analysis of variance [18] and the treatment means were compared relative to control following MSTAT C, Version 1.2 analytical software. Least Significant Differences (LSD) was calculated for mean separation.

RESULTS

Properties of Soil of the Experimental Site: The experimental soil was silty-loam and slightly alkaline in reaction, low in available P, N and medium in organic carbon. Some of the selected physico-chemical properties of the experimental site are shown in Table 1.

Effect of R. Leguminosarum on Grain Nitrogen Uptake: Inoculation of *Rhizobium leguminosarum* strain EAL 302 showed significantly ($P < 0.05$) higher grain N uptake compared to EAL 300, uninoculated and strain combination.

This might be attributed to the effectiveness of native population in colonizing the root to fix nitrogen. Field pea genotypes also affected significantly grain nitrogen uptake. Among the tested varieties, the Wayitu and Local were observed to have the highest N grain uptake than the Dadimos (Table 2).

Straw Nitrogen: Interaction effect of *rhizobial* strains with varieties showed significant variation in straw nitrogen uptake (Table 3). Uptake of N in straw increased significantly due to interaction effect of strain EAL 302 with the local variety. This might be attributed to the high nitrogen fixing ability of the introduced strain and assimilation to increase the total biomass for the available nutrient.

Total Nitrogen Uptake: Analyses of results revealed that only *rhizobial* inoculation significantly ($P < 0.05$) affected the total nitrogen uptake of field pea (Table 2). Accordingly, inoculation with EAL 302 resulted in the maximum total nitrogen uptake (169.7 kg Nha^{-1}), which was significantly high than the control. The increase in total nitrogen uptake by *rhizobial* inoculation could be the result of an increase in nitrogen fixation. In this study, it was observed that increase in total nitrogen uptake from inoculation of EAL 302 account for about 11.5% higher than the control.

Effect of R. Leguminosarum on Grain P Uptake: Inoculation of *rhizobium* strain EAL 302 significantly ($P< 0.05$) influenced grain P uptake (Table 4). The response of varieties on grain P uptake indicates that,

Table 1: Some physical and chemical properties of the experimental surface soil (0-30cm) before sowing

Parameters	Value
pH in water (1:2.5)	7.7
Organic carbon (%)	1.87
Total N (%)	0.12
Available P (ppm), Olsen	7.4
CEC (cmol.(+) kg $soil^{-1}$)	51
Na^+ (cmol.(+) kg $soil^{-1}$)	0.12
K^+ (cmol.(+) kg $soil^{-1}$)	2.4
Ca^{++} (cmol.(+) kg $soil^{-1}$)	30.9
Mg^{++} (cmol.(+) kg $soil^{-1}$)	8.2
Base saturation (%)	82
Bulk density (gcm^{-3})	1.07
Sand (%)	43.60
Silt (%)	45.48
Clay (%)	10.92
Textural class	Silty-loam

Table 2: Uptake of nitrogen as influenced by pea genotypes and *rhizobium* strains

	Nitrogen uptake ($kgha^{-1}$)		
Treatment	Straw	Grain	Total N uptake ($kgha^{-1}$)
Uninoculated	58.7	91.53	150.2
EAL 300	63.4	67.15	130.6
EAL 302	70.7	99.04	169.7
EAL 300+ EAL 302	61.6	89.05	150.7
LSD (5%)	NS	10.7	16.8
SE (±)	3.54	3.63	5.79
Local	68.0	86.97	154.9
Wayitu	59.4	95.04	154.4
Dadimos	63.5	78.06	141.5
LSD (5%)	NS	9.2	NS
SE (±)	3.07	3.15	5.01
CV (%)	16.7	12.6	11.6

NS= statistically non-significant difference between treatments

Table 3: Uptake of nitrogen in straw ($kgha^{-1}$) as influenced by genotype-*rhizobium* interaction

	Rhizobium strains			
Varieties	Uninoculated	EAL 300	EAL 302	EAL 300 + EAL 302
Local	56.38	65.02	85.29	65.12
Wayitu	47.9	69.79	69.4	50.47
Dadimos	71.82	55.41	57.37	69.25

LSD (5%) = 17.98 CV (%) = 16.7 SE (±) = 6.13

Table 4: Phosphorus uptake as influenced by pea genotypes and *rhizobium* strain

	Phosphorus uptake ($kgha^{-1}$)		
Treatment	Straw	Grain	Total P uptake ($kgha^{-1}$)
Uninoculated	2.88	5.81	8.68
EAL 300	2.82	5.46	8.28
EAL 302	4.20	6.67	10.87
EAL 300+ EAL 302	2.83	6.28	9.10
LSD (5%)	0.43	0.85	0.92
SE (±)	0.15	0.29	0.31
Local	2.96	6.04	8.99
Wayitu	3.45	6.62	10.07
Dadimos	3.14	5.51	8.65
LSD (5%)	0.37	0.74	0.8
SE (±)	0.13	0.25	0.27
CV (%)	13.7	14.4	10.2

NS= statistically non-significant difference between treatments

Table 5: Total nitrogen content of the soil after harvest as affected by *rhizobium* strain and pea genotypes

Treatment	Total N (%)
Uninoculated	0.14
EAL 300	0.11
EAL 302	0.16
EAL 300+ EAL 302	0.13
LSD (5%)	0.03
SE (±)	0.0075
Local	0.12
Wayitu	0.15
Dadimos	0.13
LSD (5%)	0.03
SE (±)	0.0065
CV (%)	10.7

NS= statistically non-significant difference between treatments

Table 6: Nitrogen uptake by the reference crop (barley) and post harvest N from reference crop field

Parameters	Value ($kgha^{-1}$)
Uptake of N in grain	19.8
Uptake of N in straw	15
Total N uptake	34.8
Total N (after harvest)	111.1

Wayitu variety (6.62 kg Pha^{-1}) performed better than other varieties tested. An increment of 17% in grain P uptake was observed in Wayitu compared to Dadimos variety.

Straw Phosphorus: P uptake in straw was affected by both rhizobium strains and pea genotypes. P uptake in the straw significantly ($P< 0.05$) increased due to inoculation of EAL 302 compared to control (Table 4). The highest straw P uptake on the other hand, was observed with Wayitu (3.45 kg Pha^{-1}) comparable to P uptake with Dadimos (3.14 kg Pha^{-1}). There was no interaction effect for straw P uptake between varieties and strains.

Total Phosphorus Uptake: The maximum total phosphorus uptake was observed in strain EAL 302 (10.87 kg Pha^{-1}) which was significantly high compared to the control. Varietal response to total P uptake showed the highest total P with Wayitu genotype 11% higher than local genotype. In this study, total P uptake was comparable to the total amount of N fixed indicating that microbial activity in terms of N fixation was highly dependent on the availability of P.

Effect on Residual Nitrogen after Harvest: The post harvest total nitrogen soil analysis showed both the introduced strains and varieties markedly affected ($P< 0.05$) nitrogen in soil. The highest total nitrogen was noticed due to EAL 302 (0.16%) and control (0.14%) which was significantly higher than EAL 300 (Table 5). This showed that indigenous *rhizobia* contribute to the total N in the soil.

Pea varieties especially Wayitu (0.15%N) contributed significantly high amount of N to soil compared to Dadimos and local. This indicates that pea fixing ability of crop may contribute more nitrogen to the succeeding crop.

Table 7: Amount of symbiotically fixed N ($kgha^{-1}$) contributed by *rhizobium* strains in pea varieties

	Rhizobium strains				Mean
Varieties	Uninoculated	EAL 300	EAL 302	EAL 300+302	
Local	111.97	96.34	153.87	118.31	120.12
Wayitu	113.39	105.8	146.91	112.45	119.64
Dadimos	120.93	85.13	104	116.83	106.72
Mean	115.43	95.76	134.93	115.86	115.49
	Strain	Variety		Interaction	
SE (±)	5.79	5.01		10.03	
LSD (0.05%)	16.98	NS		NS	
CV (%)			15.04		

* Total N uptake in reference crop, barley = 34.8 $kgha^{-1}$

NS= statistically non-significant difference between treatments

Table 8: Amount of symbiotically fixed N ($kgha^{-1}$) left in the soil

	Rhizobium strains				Mean
Varieties	Uninoculated	EAL 300	EAL 302	EAL 300+302	
Local	71.3	-9.26	120.19	60.19	60.61
Wayitu	118.34	60.19	129.63	100	102.04
Dadimos	61.48	55.56	71.48	60.19	62.18
Mean	83.71	35.5	107.1	73.46	74.94
	Strain	Variety		Interaction	
SE (±)	10.47	9.06		18.14	
LSD (0.05%)	30.71	26.6		NS	
CV (%)			14.92		

* Total soil N in reference crop, barley at physiological maturity = 111.11 $kgha^{-1}$

NS= statistically non-significant difference between treatments

Table 9: Amount of N-fixed as affected by *rhizobium* strain and field genotypes

Treatment	N in legume ($kgha^{-1}$)	N in soil ($kgha^{-1}$)	N yield ($kgha^{-1}$)
Uninoculated	115.43	83.71	199.1
EAL 300	95.76	35.5	131.2
EAL 302	134.93	107.1	242.0
EAL 300+ EAL 302	115.86	73.46	189.3
LSD (5%)	16.98	30.71	37.04
SE (±)	5.79	10.47	12.6
Local	120.12	60.61	180.7
Wayitu	119.64	102.04	221.7
Dadimos	106.72	62.18	168.9
LSD (5%)	NS	26.6	32.08
SE (±)	5.01	9.06	10.9
CV (%)	15.04	14.92	19.89

NS= statistically non-significant difference between treatments

Effect of Treatments on Biological Nitrogen Fixation: It was observed that inoculation of *rhizobial* strains affected the fixed nitrogen considerably. The non-fixing reference crop barley, uptake of N for the determination of N-fixed as a result of symbiotic nitrogen fixation was presented in Table 6.

Thus, the total nitrogen fixed symbiotically derived by legume crop as well as the one left in the soil was calculated using the following equation:

$$Q = [N \text{ yield (leg)} - N \text{ yield (ref)}] + [N \text{ soil (leg)} - N \text{ soil (ref)}]$$

From the results obtained, except EAL 300 all other inocula contributed the fixation of nitrogen. Maximum fixed nitrogen was due to inoculation with EAL 302 (242 kg N ha^{-1}) though strain combination and the control also contributed to the fixed N respectively. Variations among pea genotypes for the fixed nitrogen were also observed; Wayitu fixed 19% of nitrogen compared to local and Dadimos varieties and this indicate the potential of improved varieties for nitrogen fixation. Also, the study showed an increment of the fixed nitrogen by 46% and 18% than EAL 300 and control respectively to *rhizobial* strain EAL 302 indicating the necessity of efficient nitrogen fixing *rhizobium* strains and good inoculation practice in pulse production.

DISCUSSION

The result of soil analysis indicates that the soil of Sinana was low in total nitrogen, available phosphorus but moderate in organic carbon. This agreed with the previous work of Asnakew *et al.* [19] who observed that poor soil fertility, especially low levels of nitrogen and phosphorus, is a major constraint to crop production in Ethiopia. These are ideal conditions for providing rhizobial association with the pea roots in order to exploit the potentials of micro organisms in maintaining soil fertility. Rhizobial inoculation results of this study confirm the findings Yifru [20] who reported the increased total nitrogen uptake as a result of inoculation of *Rhizobium leguminosarum*. The uptake of N in straw also increased significantly due to interaction effect of strain EAL 302 with local variety which might be attributed to the high nitrogen fixing ability of the introduced strain and its assimilation by the crop enhancing the total biomass as observed by Chabot [21]. Studies conducted to evaluate the performance of different lentil varieties showed the synergistic relationships among inoculated plots with *rhizobium* strain than uninoculated treatments for their high protein content and nitrogen uptake [22]. Correspondingly, research report on soybean at field condition using *Bradyrhizobium* inoculation confirmed the significant positive effect on N uptake by the shoot [23].

Inoculation of *rhizobium* strain with EAL 302 appreciably influenced grain P uptake than the uninoculated. It was noticed that strain EAL 302 showed higher N_2-fixation. In a similar study carried out on P requirements for N_2-fixation in pea (*Pisum sativum*) revealed P requirements are generally higher for N_2-fixation [24]. Varietal responses, on the other were also observed for their effectiveness in nitrogen fixation and, hence, high uptake of P and this was in agreement with the report of Mefti *et al* [25]. Such response according to Israel [26] affects nitrogen dependent legumes as symbiotic nitrogen fixation is an energetically expensive process. In this study total P uptake was comparable with the total amount of N fixed indicating the highly dependent nature of microbial activity on the availability of P in terms of N fixation.

The residual soil nitrogen as a result of inoculation of *rhizobial* strain markedly increased over uninoculated this confirm the assertion of Myers and Wood [5] that the contribution of N_2 fixing plant to the sustainability of agricultural system depends on how much of the fixed N is harvested and removed from the soil. Significant variability in post harvest total soil nitrogen was observed among varieties and this corroborates the findings of Ravuri and Hume [27] who maintained that cultivar-strain interactions contribute considerably to the amount of fixed N_2 under field conditions. As a result, response from variety Wayitu for the fixed N was highly significant than other varieties studied. Also FAO [28] reported in this regard the variability that exists among pulse crops to the amount of fixed N. Similarly, Peoples *et al.* [2] and Brockwell *et al.* [6] also reported the tremendous potential for fixed N contribution in soil among legumes. In agreement with the given study, research findings had proved *rhizobial* inoculation brought a significant effect on nutritional value from inoculated treatments than uninoculated treatments. This could be related to the nitrogen fixation ability of nodules, which consequently results in increased nutrient uptake, growth and yield [29]. Similar results are indicated by many workers [30-32].

CONCLUSION

The loss of soil fertility is becoming a serious problem to nation's food security program and one way to combat such a threat is through incorporating beneficial soil organisms both for soil fertility build up and boost crop yield simultaneously. It is imperative to make use of appropriate artificially produced strains for the majority of poor and/or less productive zones of tropical soils. Based on the findings of these study it was noticed that strain EAL 302 on Wayitu pea variety produced the best combination with respect to response in terms of nutrient uptake and fixed N in soils of the study area. Thus, further field studies in respect of other rhizobial species for comparison and evaluation as they increase soil fertility and trials of combination of different inocula needs attention for sustainable crop production.

ACKNOWLEDGEMENT

The authors acknowledged gratefully Oromia Agricultural Research Institute in financing the project. The support given by Sinana Agricultural Research Center is also appreciated.

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