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Measurement of Competitive Ability of *Rhizobium leguminosarum* in Different Pea Genotypes under Sterilized and Unsterilized Soil Conditions

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Abstract: A study to examine the effects of genotype x *Rhizobium leguminosarum* strain interaction for their effectiveness under green house condition both in sterilized and unsterilized conditions was conducted in soils of Sinana in 2005. Two strains, EAL 300 and EAL 302 of *Rhizobium leguminosarum* and their combination were evaluated for their interaction with three varieties of field pea grown under green house in terms of nodulation parameters. Pot trial conducted in green house under sterilized soil showed *rhizobial* strains and pea varieties significantly affected nodule number, nodule dry weight and plant dry matter. Maximum nodule number was reported from strain EAL 302 (28) and variety 'Wayitu' (21). EAL 302 increased plant dry matter by 15% over uninoculated whereas plant dry matter affected by variety 'Wayitu' increased 14% over local. In unsterilized soil condition interaction of variety and strains significantly affected the nodule number while nodule dry weight was affected by strains and varieties. On the other hand, plant dry matter was only affected by field pea varieties. Thus, based on the findings it could be suggested that the interaction effect observed in unsterilized soil than the sterilized for nodule number and plant dry matter promote the presence of best combination of genotype x *rhizobium* interaction with respect to response in terms of nodulation and N-fixation.

Key words: interaction · Nodulation · Strain · Variety · Fixation

INTRODUCTION

Inoculation of legumes with *rhizobium spp.* ensures nodulation and efficient nitrogen fixation. Historical events revealed that the soil enriching quality of leguminous crops was known during the time of the Roman Empire when rotational cropping systems were introduced.

With the isolation of the causal organism, *rhizobium*, by Beijerinck in the late 19th century, microbiologists and agronomists began to devise more efficient ways of achieving inoculation of seeded legumes[1]. However, host legumes are influenced by means of rhizosphere effects, which to a great extent modify the distribution and population size of *rhizobia* [2].

The legume that may be nodulated sometimes resulted in the fixed nitrogen production below the level of the required for optimal growth of the host plant. This is merely due to the low and/or inefficient native population present in the soil. In such cases, symbiosis association may better adapted or more adaptable for insitu management at sufficient levels to assure contact with susceptible legumes roots Li and Alexander; Pugashettie *et al.* Ramirez and Alexander [3-5]. So the population of the nodules occupied by inoculated *rhizobial* strains depended on the population of the indigenous soil organisms [6].

Symbiotic nitrogen fixation rates could be markedly increased by highly efficient, competitive and persistent strains of *rhizobia* [7] and by optimizing crop management practices such as those related to sowing time, insect pest and weed control and phosphorus nutrition [8]. With this general remark the study was conducted to evaluate the effectiveness of the introduced strains on one hand and the efficiency of the native *rhizobia* on the other.

MATERIALS AND METHODS

The required amount of a bulk soil sample from the field was taken for conducting pot trial in the green house both under sterilized and unsterilized soil condition.

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The soil was autoclaved at 121°C for 2 hrs for three consecutive days. The sterilized soil was filled in separate plastic pots at the rate of 4 kg/pot after washing their surface with alcohol.

Seeds of field pea varieties local, 'Wayitu' and 'Dadimos' were surface sterilized by successive treatment of 0.1% $HgCl_2$ solution and absolute alcohol and then 4 -5 washings with sterilized water. Surface sterilized seeds were inoculated with various strains and their combination @ 7 g/kg seed. The pots were arranged in completely randomized block design with two replications for each treatment both under sterilized and unsterilized soil conditions. Starter dose of fertilizer, 20 kg Nha^{-1} as urea and 46 kg $P_2O_5ha^{-1}$ as TSP, was applied to each pot. Three plants per pot were maintained for each replication and six weeks after planting, the plants were carefully uprooted and examined for nodulation parameters and plant dry matter.

Treatments and Design: Pot experiment on a silty-loam soil sampled from a representative farmer's field was laid out in factorial RCBD with 12 treatments and two replications as described below:

Inoculant strains	Pea Varieties
A. Uninoculated (control)	A. Local
B. EAL 300	B. 'Wayitu'
C. EAL 302	C. 'Dadimos'
D. EAL 300 + EAL 302	

Observations

Nodulation: Sampling for nodulation was performed by excavating the roots of the remaining three plants in the pot. The plant samples were wrapped in plastic and transported to the laboratory where the soil was washed from the roots using gently running tap water. Care was taken to ensure that the roots and nodules were recovered intact. Nodules from crown region and lateral roots subsequently were removed separately from the roots. The total number of nodules were counted and the mean value of the three plants was recorded as number of nodules per plant. The collected nodules were dried in an oven to a constant weight to determine nodule dry weight per plant. The average of three plants was taken as nodule dry weight per plant.

Plant Dry Matter: Plant dry matter was determined from plants sampled for nodulation. After the nodules had been collected from roots, the plant samples were placed in a labeled perforated paper bags and oven-dried to a constant weight to determine the plant dry matter. The average dry weight of three plants was measured to determine dry weight per plant.

Physico-Chemical Analysis of Soil: Samples were analyzed for the following parameters *viz.,* bulk density, particle size distribution, pH, organic carbon, cation exchange capacity, exchangeable bases (Na, K, Ca and Mg), base saturation, total nitrogen and available P from the representative bulk soil sample before planting. Total N was analyzed for each treatment in all replications following the standard procedure after harvest.

Soil bulk density was estimated by core method and soil pH by potentiometric method at soil: water ratio of 1:2.5 [9]. Cation exchange capacity was determined by 1M ammonium acetate method at pH 7 [10] whereas organic carbon was determined by the dichromate oxidation method [11] and total nitrogen by the micro kjeldhal method Jackson [12], available P was analyzed by Olsen method [13] and determined colorimetrically by the ascorbic acid- molybdate blue method [14, 13]. Ca^{++} and Mg^{++} values were found out from Atomic Absorption Spectrophotometer reading while Na^+ and K^+ was determined using flame photometer. Soil particle size distribution was determined by hydrometer method [15].

Statistical Analysis: Data were analyzed using analysis of variance [16] and the treatment means were compared relative to control following MSTAT C analytical software. Least Significant Differences (LSD) was calculated for mean separation.

RESULTS

Properties of Soil of the Experimental Site: The experimental soil was low in available P, which indicated the need for phosphorus application. Such deficient soil therefore may have apparently influenced the microbial activity since phosphorus plays important role in several energy requiring biochemical reactions including biological nitrogen fixation. Usually these two nutrients are often limiting crop production in Ethiopia. Considering other parameters, the experimental site has very high in cat ion exchange capacity, medium in organic carbon content and very high in base saturation. The base-forming component (exchangeable base) was very low in exchangeable Na^+ in particular and the exchange site is highly dominated by Ca^{++}. Some of the selected physico-chemical properties of the experimental site are shown in Table 1 below.

Effectiveness in Sterilized Soil: The main effect of *rhizobial* strains EAL 302 gave the highest nodule number, nodule dry weight and plant dry matter as compared to uninoculated control which did not show any nodule. Likewise, variety 'Wayitu' significantly showed increased nodule number, nodule dry weight and plant dry matter over local and 'Dadimos'.

The interaction effect shown in Tables (3 and 4) affected both nodule number and nodule dry weight. Maximum nodule number was scored when variety 'Wayitu' was inoculated with EAL 302 and 'Wayitu' with EAL 300 both of which were statistically at par. Similarly, the interaction effect observed in 'Wayitu' x EAL 302 and 'Wayitu' x EAL 300 resulted in maximum nodule dry weight statistically at par. The results indicated both the strains were capable of nodulating different varieties of field pea in absence of native *rhizobia*, however, to varying degrees. Similar trend was observed in relation to dry matter of plants where inoculation of EAL 302, as compared to EAL 300, resulted in significantly higher dry matter in different varieties grown in sterilized soil (Table 2).

Effectiveness in Unsterilized Soil: Pot trial conducted in unsterilized soil condition showed *rhizobial* strain and variety didn't affect nodule number. However, the interaction effect of strain and variety significantly ($P \leq 0.05$) affected nodule number (Table 6). Maximum number of nodules was recorded from interaction effect of EAL 302 on local variety which was statistically at par with 'Wayitu' inoculated with EAL 302. The least number of nodules were scored due to local variety under uninoculated conditions indicating the need for inoculation. Very low number of nodules in uninoculated plot might be associated with the mono cropping system which didn't allow the actual size of microbial population or less infectivity of native population. On the other hand, the interaction and sole variety effect had marked influence on the plant dry matter but introduced strains didn't affect plant dry matter (Table 7).

The present green house study measured the nodule dry weight per plant in unsterilized soil sample. The result of analysis of variance showed strains and varieties significantly ($P \leq 0.05$) affected nodule dry weight. The highest nodule dry weight per plant was recorded in strain EAL 302 and variety 'Wayitu' (Table 5).

In general, the results of green house experiment under sterilized and unsterilized soil conditions indicated the higher N fixing capacity (as reflected by more nodulation and dry matter of plants) and competitiveness (as reflected by more nodulation in unsterilized soil) of strain EAL 302 as compared to EAL 300 and native *rhizobia*.

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

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

Effectiveness of *Rhizobial* Strains under Green house Conditions

Table 2: Nodulation and dry matter yield/ plant at mid flowering stage as affected by *rhizobium* strains and pea varieties in sterilized soil in green house condition

Treatment	Nodule no./plant	Nodule dry wt./plant (mg)	Dry matter/ plant (g)
Uninoculated	0	0	1.915
EAL 300	23	44.67	2.257
EAL 302	28	53.17	2.407
EAL 300+ EAL 302	19	18.50	2.160
LSD (5%)	2.81	4.14	0.23
SE (±)	0.9	1.33	0.07
Local	16	16.00	2.041
Wayitu	21	56.38	2.374
Dadimos	15	15.13	2.139
LSD (5%)	2.44	3.58	0.19
SE (±)	0.78	1.15	0.06
CV (%)	12.7	11.2	8.3

NS= statistically non significant difference between treatments

Table 3: Interaction effect of strains and varieties on nodule number in sterilized soil in green house condition

	Rhizobium strains			
Varieties	Uninoculated	EAL 300	EAL 302	EAL 300 + EAL 302
Local	0	16	28	21
Wayitu	1	36	38	11
Dadimos	1	19	18	24

LSD (5%) = 2.435 CV (%) = 12.7 SE (±) = 1.56

Table 4: Interaction effect of strains and varieties on nodule dry weight (mg) in sterilized soil in green house condition

	Rhizobium strains			
Varieties	Uninoculated	EAL 300	EAL 302	EAL 300 + EAL 302
Local	0	16	27.5	20.5
Wayitu	0.5	99.5	114.5	11
Dadimos	0.5	18.5	17.5	24

LSD (5%) = 7.163 CV (%) = 11.2 SE (±) = 2.3

Table 5: Nodulation and dry matter yield/ plant at mid flowering stage as affected by *rhizobium* strains and pea varieties in unsterilized soil in green house condition

Treatment	Nodule no./plant	Nodule dry wt./plant (mg)	Dry matter/ plant (g)
Uninoculated	20	61.83	2.421
EAL 300	21	43.33	2.713
EAL 302	24	82.33	2.865
EAL 300+ EAL 302	22	57.00	2.530
LSD (5%)	NS	21.9	NS
SE (±)	0.95	7.05	0.11
Local	22	58.13	2.537
Wayitu	23	77.75	3.234
Dadimos	20	47.50	2.127
LSD (5%)	NS	19.0	0.30
SE (±)	0.82	6.12	0.1
CV (%)	10.8	28.2	10.6

NS= statistically non significant difference between treatments

Table 6: Interaction effect of strains and varieties on nodule number in unsterilized soil in green house condition

	Rhizobium strains			
Varieties	Uninoculated	EAL 300	EAL 302	EAL 300 + EAL 302
Local	16	23	27	22
Wayitu	20	20	27	24
Dadimos	23	20	17	20

LSD (5%) = 5.133 CV (%) = 10.9 SE (±) = 1.65

Table 7: Interaction effects of strains and varieties on plant dry matter (g) in unsterilized soil in green house condition

	Rhizobium strains			
Varieties	Uninoculated	EAL 300	EAL 302	EAL 300 + EAL 302
Local	2.283	2.780	2.775	2.310
Wayitu	2.985	2.675	3.895	3.380
Dadimos	1.995	2.685	1.925	1.901

LSD (5%) = 0.615 CV (%) = 10.6 SE (±) = 0.2

DISCUSSION

The study perfectly assesses the potential competitive ability of strains in producing effective nodules which fixes appreciable amount of atmospheric nitrogen in green house. As a matter of fact *rhizobium* is known to survive in soil and in the rhizospheres of legumes as well as in non-legumes. There is evidence to support that the bacterium thrives on root excretions although no single component in the root exudates have any special role in stimulating its growth [17]. Accordingly, the study on the effect of nodulation and plant dry matter revealed strain EAL 302 was superior both in sterilized and unsterilized pot trial as compared to EAL 300 or combination of strains (EAL 300 + EAL 302). In sterilized condition in which native *rhizobia* removed and no competition exists, strain EAL 300 was highly competitive with strain EAL 302. However, this effect was not observed under unsterilized soil condition. Therefore, the presence of *rhizobia* is not always sufficient, in itself; to ensure optimal nitrogen-fixing capacity in the host legume as the effectiveness of strains to fix nitrogen within naturalized populations can vary considerably. It is usually accepted that it is difficult to introduce superior strains (by inoculation) when there is a high background population of indigenous and well-adapted *rhizobia* and nodules occupied by the inoculant strain may decline in the years following inoculation [18-21].

The native indigenous populations in most of the parameters studied under unsterilized green house condition were better as compared to EAL 300. The variation in response to inoculation from varieties involved was also promising. Mytton and Skot [22] reported the positive interaction effect and response of the legume host and *rhizobium* strain in *Vicia faba* for nodulation. Variety 'Wayitu' was positively responded and highly expressed its genetic potential in most of the interactions effect and solely variety responses. As an important remark the legume that may be nodulated sometimes resulted in the fixed nitrogen production below the level of the required for optimal growth of the host plant. This is merely due to the low and/or inefficient native population present in the soil. In such cases, symbiosis association may better adapted or more adaptable for insitu management at sufficient levels to assure contact with susceptible legumes roots [3-5]. So the population of the nodules occupied by inoculated *rhizobial* strains depended on the population of the indigenous soil organisms [6].

It is highly recommended to delineate the resistance of strains to the various antibiotics facing in the complex soil environment and genetic potential of genotypes in agricultural sector. On the basis of the findings it is therefore, strain 302 and variety 'Wayitu' were the best as compared to the rest.

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