Original Research Article

Symbiotic effectiveness of indigenous rhizobial strains on biological nitrogen fixation of Lablab (*Lablab purpureus*) in the derived savanna of Nigeria

Abstract

Field and pot experiments were conducted in the derived savanna of Nigeria to determine the effectiveness of three indigenous rhizobial strains on the Nitrogen (N) fixation of lablab (*Lablab purpureus*). Soils were collected from two locations, Idi Ayunre and University of Ibadan Teaching and Research Farm (UITRF) for pot experiment in a completely randomized design with a factorial arrangement of 2 × 2 × 6. The treatments were soil type (Idi Ayunre and UITRF soils), sterilization (partially-sterile and unsterile) and six rhizobial strains inoculation {three indigenous strains, IDC8, OISa-6e and TRC; two exotic strains, IRj 2180A and R25B; and the control (resident native rhizobial strains)}. Field experiment was further conducted at UITRF using five rhizobial inoculations, the three indigenous strains, combination of R25B and IRj 2180A (R25B+IRj 2180A) and control. Data were collected on biomass dry weight, number of nodules, nodule dry weight, N derived from the atmosphere (Ndfa), N and P uptake and total N fixed. No significant difference was observed in the Ndfa (%) among the strains. However, indigenous strains IDC8 and OISa-6e performed better than the resident native rhizobia in terms of N fixed, biomass dry weight, nodule formation and N uptake. N fixed by lablab inoculated with IDC8 was more than 200% higher than that fixed by resident native bacteria. Lablab N fixation efficiency can be improved using effective indigenous rhizobial strains. Further screening of indigenous strain for N fixation efficiency is recommended.

Keywords: Rhizobium N fixation Forage legume; Lablab

Introduction

The soil improvement potential of lablab (*Lablab purpureus*) has continued to generate research attention in the derived and moist humid savannah of West Africa where soil fertility has continued to decline due to the pressure caused by increasing population. Lablab is widely cultivated in sub Saharan Africa and like other food and forage legumes, it possesses qualities that could prove exceptionally valuable for Africa's rural development and environmental stability (Adebisi and Bosch 2004). These qualities include wide adaptation to different environment, ease of planting and management under subsistence production typical of African agriculture, high yield, drought tolerance and soil improvement potentials (Adebisi and Bosch 2004; Cook et al. 2005; Nworgu and Ajayi 2005, National Research Council 2006; Maass et al. 2010). Sanginga et al. (1996) reported that *Lablab purpureus* had a high N_2-fixing capability and could also adapt to low-P soils in the Guinea savanna of West-Africa.

The high cost and environmental risks associated with the use of chemical fertilizer has made lablab cultivation one of the alternatives for low cost and sustainable N supply in the soil. Lablab can produced about six tonnes of total biomass containing about 140 kg N which can be returned back to the soil after decay (Valenzuela and Smith 2002; FAO 2012). Mcdonald et al. (2001) estimated N_2 – fixed by lablab to be 177 kg N ha^{-1} which to an extent is substantial for soil fertility improvement. Lablab inclusion into cereal-legume based cropping systems especially maize based production system as an intercrop or as fallow crop has yielded beneficial results in terms of crop yields and soil fertility improvement (MacColl 1990; Tarawali 1991; Devkota and Rerkasem, 2000). Apart from the N-fixation potential, lablab as a cover crop is able to conserve soil and compete with weeds (Schaaffhausen 1963) thereby improving the soil quality for crop production.

Nitrogen fixation in all legumes is due to their symbiosis with the soil bacteria rhizobium. However, the amount of atmospheric N that is fixed is partly hinged on the effectiveness of the rhizobial strains that infect the plant for nodulation and fixation. Biological nitrogen fixation is a natural process, which can be improved by introducing or inoculating legumes with an efficient rhizobia strain for effective nitrogen fixation. FAO (2012) suggested that the seed of lablab should be inoculated with a cowpea-type, Bradyrhizobium strain as it does not easily nodulate with native rhizobia. However, indigenous or native rhizobia have been found to be effective for some legumes like cowpea in some parts of West Africa (N2Africa 2013). Efficient rhizobial inoculants could be obtained by isolating, purifying and screening the native or indigenous rhizobia with standard rhizobia under controlled conditions (Kumar et al. 1997). In Nigeria, Ojo et al (2015) isolated three rhizobial strains from derived savanna soils that are comparable in infectivity and nodulation with some introduced/exotic strains. Thies et al. (1991) reported that the success of any rhizobial inoculation starts with the ability of the inoculant strain to survive and nodulate the host plant.

Legume-rhizobial symbiosis is an efficient source of soil nitrogen for sustainable agriculture. Since lablab is among the promising forage legumes that have been identified to improve agricultural sustainability in the moist savannah (Tarawali 1991), this study investigated the effectiveness of three native rhizobial strains on biological N fixation of lablab.

Materials and Methods

Pot experiment

A pot experiment was conducted at the International Institute of Tropical Agriculture screenhouse, Ibadan, Nigeria using topsoil (0-15 cm) collected from two locations in the rainforest-savanna transition zone of Nigeria, Idi-Ayunre (latitude $7^{o}26´$N and longitude

3°54´E) and the University of Ibadan Teaching and Research Farm (UITRF, latitude 7°30´N and longitude 3°45´). Idi-Ayunre has soil classified as Nitosol and that of the University of Ibadan Teaching and Research Farm as Alfisol (USDA, 2006). The samples were analysed for pH in water (1:1) (IITA, 1982), soil organic matter using wet dichromate acid oxidation method (Nelson and Sommers, 1982), total nitrogen using Kjeldahl analytical method (Bremner and Mulvaney, 1982), available phosphorus using Bray-1 method (Bray and Kurtz, 1945), particle size using Bouyoucus hydrometer method (Okalebo et al., 1993), exchangeable Mg, Ca, K and Na extracted using neutral 1M ammonium acetate and determined with spectrophotometer (Okalebo et al., 1993).

Soil samples for each location were divided into two parts, one part was sterilized using direct flaming method with the aid of a Terraforce sterilizing machine (IITA fabricated sterilizing machine). Two kilograms soil was weighed into each pot for the planting

Rhizobial population count

The rhizobial population count of the two locations was determined using the Most Probable Number (MPN) as outlined by Somasegaran and Hoben (1994). Two soyabean varieties, TGx1448–2E and TGx 1456-2E, and one cowpea variety IT89KD-288 seeds were sterilized, pre-germinated and transplanted into sterilized growth pouches containing modified Jensen's N-free nutrient solution (Roughley, 1984). A 5-fold dilution series (5^{-1} – 5^{-6}) of the soils of the location with four replicates was used to inoculate each plant in the growth pouch one week after planting. The diluent contained 0.250 g K_2HPO_4 and 0.10 g Mg SO_4. $7H_2O$ dissolved in 1 L distilled water. Nodule formation on plants was observed and recorded for thirty days. The presence (+) or absence (-) of nodules on each plant were scored and MPN values were calculated with MPN table.

Experimental design and data collection

The experimental design was a 2 × 2 × 6 factorial arrangements in a completely randomized design with three replicates. The treatments were location (Idi-Ayunre and UITRF), soil sterilization at two levels (sterile and unsterile) and rhizobial strains at six levels (control, Isolate 1 – OISa-6e, Isolate 2 – IDC8, Isolate 3 – TRC2, IITA strain 1 – R25B, IITA strain 2 – IRj 2180A). Two seeds of lablab were planted per pot and inoculation was done one week after planting using dispenser to directly inoculate the soil just below the seedling with 2 ml broth culture of the rhizobial strains. Data were collected at harvest to determine the shoot dry weight, number of nodules per pot, nodule dry weight per pot and percentage nitrogen derived from atmosphere [Ndfa (%)]. N_2 fixed was determined by taking samples from stem + petioles for tissue extraction (Hot water extract) for ureide – N and NO_3 –N in the laboratory. The procedure was followed as outlined by Herridge (1982).

Description of rhizobial strain

The five strains that were used for the experiment were IDC8, TRC, OISa-6e, IRj 2180A and R25B. The IDC8, TRC and OISa-6e were indigenous strains isolated from cowpea planted on Idi-Ayunre and UITRF soils and soybean planted at Orile Ilugun soil (Ojo et al 2015). The IRj 2180A (soybean isolate) and R25B (promiscuous isolate) were rhizobial collections from International Institute of Tropical Agriculture, Ibadan, Nigeria (Sanginga et al 2000).

Field experiment

Field experiment was set up at UITRF in a randomized complete block design with three replicates. The rhizobial strains IDC8, TRC2, OISa-6e, R25B+IRj 2180A and the control were used as treatments. R25B and IRj 2180A were exotic strains used separately in the greenhouse experiment but mixed together for the field experiment.

One kilogramme of seeds of lablab was inoculated with 10 g peat culture of each rhizobial strain using the method of Somasegaran and Hoben (1994). The Yeast Mannitol Broth (YMB) cultures of each strain were aseptically injected into different peat package carriers at

ratio 1: 1 (ml/wt in g). Inoculated peats were incubated for 2 weeks at 28^{o}C to gain excess of 10^8 - 10^9 cells g^{-1}. Planting was done between July and early August using a late maturing variety of lablab NAFR14. The seeds were planted at a spacing of 75 cm × 25 cm.

Data collection

Plants were uprooted systematically at three points per plot using a 30 cm × 30 cm quadrant. Root of five plants and soil under the quadrant area were removed to a depth of 15 cm to determine number of nodules. The nodules on the root and those detached in the soil were counted and weighed to get the fresh nodule weight and oven-dried at 78^{o}C to a constant dry weight. The root and the shoot were separated, air-dried for 72 hours before oven dried at 78^{o}C to constant dry weights. The stem + petioles of the five sampled plants were and ground. Ureide – N and NO_3 – N were determined from the stem + petioles samples using tissue extraction (hot water extract) as described by Herridge (1982). Shoot biomass N and P contents were also analysed using the method of Herridge (1982). Harvesting was done at physiological maturity of each variety.

Statistical analyses: Data obtained were subjected to analysis of variance using PROC GLM of SAS (SAS 2003). Data for number of nodule were transformed using square root transformation. Means were separated at a significant level P = 0.05 using Least Significant Differences (LSD).

Results

Physical, chemical and biological properties of the experimental soils

The Idi Ayunre and UITRF soils were slightly acidic with UITRF being more acidic. The total N, available P, Ca, Mg and Na were higher in Idi-Ayunre soil compared to UITRF soil. In fact, total N and available P were more than two-fold higher in Idi-ayunre soil compared to those of UITRF soil (Table 1). The soil physical properties of Idi-Ayunre soil revealed a

sandy loam soil while that of UITRF revealed a loamy sand soil. The number of rhizobial cell in the UITRF soil was significantly higher (P=0.05) than that of Idi Ayunre (Fig. 1). The number of rhizobial cell (14.2 $\times 10^6$ g-1 soil) in the UITRF was about two-fold higher than that of Idi Ayunre (7.3 $\times 10^6$ g-1 soil).

Growth and nodulation of lablab in pot experiment

The soils used for the pot experiment significantly (P=0.05) affected biomass dry weight, number of nodules, nodule dry weight and Ndfa (%). The biomass, nodule dry weights and Ndfa (%) were significantly higher (P=0.05) in Idi Ayunre soil than UITRF soil (Table 2). However, the numbers of nodules were higher in UITRF soil than Idi Ayunre soil. The effect of soil sterilization was significant on nodulation as partially sterile soil had significantly lower (P=0.05) number of nodules and nodule dry weight compared to unsterile soil. However, biomass dry weight was significantly higher (P=0.05) in sterile soil than unsterile soil. The rhizobial strains used for the inoculation of lablab in the pots significantly affected nodulation of the plants. The numbers of nodules formed by lablab in the pot were significantly higher in inoculated plants than the control (Table 2). Similarly the nodule dry weight was higher in inoculated plants than the uninoculated (control). The indigenous strain IDC8 isolated from Idi Ayunre soil had the highest number of nodules followed by the exotic strain R25. The effect of the rhizobial strains on nodulaton did not reflect in the Ndfa (%) as there was no significant difference between inoculated plants and uninoculated plants (Table 2).

The effect of interaction of the soil, sterilization and inoculants on nodulation revealed that numbers of nodules of lablab in UITRF unsterile soil was significantly higher than the number of nodules in the sterile soils and unsterile of Idi-Ayunre (Table 3). In both sterile and unsterile soil of UITRF, the number of nodules in inoculated plants was significantly higher than the control plants except the plants inoculated with IRJ 2180A and

OISa-6e in sterile soil. The number of nodules of plants under unsterilized soil inoculated with R25 and TRC2 in Idi-Ayunre and IDC8 and IRJ 2180A in UITRF were significantly higher than their corresponding treatment in sterile soil. In sterile soil at Idi-ayunre and UITRF, IDC8 inoculated plants had significantly higher number of nodules than the uninoculated plants. The nodule dry weight of the unsterile soil in Idi-Ayunre was significantly higher than that of sterile soils in the two soil type and that of the unsterile soil in UITRF (Table 3). Inoculated plants with bacterial strains OISa-6e, R25 and TRC2 in Idi Ayunre had significantly higher nodule dry weight than the control plant. In UITRF, only IDC8 had higher nodule dry weight than the control treatment. In spite of significantly higher number of nodules in UITRF when inoculated plants in sterile were compared to the control, the nodule dry weight were not significantly difference (Table 3).

Growth, nutrient uptake and N fixation of lablab at UITRF (field experiment), Ibadan

The biomass dry weights of lablab in the plots inoculated with the strains IDC8 and R25 were significantly higher than those of the strain TRC3 and the control. In fact, there was more than two-fold increase in the biomass dry weight of lablab inoculated with IDC8 than that of the control (Fig 2). The number of nodule in lablab inoculated with indigenous rhizobial OISA-6E and IDC8 were significantly higher than the other treatments (Table 4). The control plant had the lowest number of nodules but was not significantly lower than plants inoculated with TRC2. There was no significant difference in the Ndfa (%) when the rhizobial strains were compared even with the control. However, the total N fixed (kg/ha) in the IDC8 inoculated plants was significantly higher than other strains except R25B+ IRj2180A inoculated plants. The control treatment had significantly lower total N fixed when compared with other strains except TRC2. The N fixed (kg/ha) in IDC8 and R25B plots was about 144% and 93% higher than control plot respectively (Table 4). The N and P contents of plant and P uptake were not significantly affected by the inoculation of rhizobial strains. However,

199 the N uptake was significantly increased in IDC8 inoculated plants compared to the control
200 and TRC2 inoculated plants (Table 5) .

201 **Discussion**

202 The biological analyses of the bacteria cells in the two soils revealed a very low population of
203 rhizobial cells with UITRF having higher population which almost double that of Idi Ayunre.
204 The rhizobial count observed was within the range of 2×10^0 to 3.2×10^3 reported by
205 Abaidoo et al., (2007) for twelve sites in Nigeria. Soils that have never been inoculated or
206 cultivated with legumes were selected for the study and this probably contributed to the low
207 rhizobial cell count. Slattery and Pearce (2002) also observed a range varying from less than
208 10 to 10^6 in Australian soils and reported that considerable variation exist between bacteria
209 population in soils and could be due to several factors which include field history, location of
210 sampling, soil characteristics and the presence of a host plant. The two locations had different
211 soil characteristics and field history. Idi Ayunre soil was under bush fallow of over ten years
212 unlike UITRF soil which was collected from a research farm cultivated with crops others than
213 legumes. The detection of rhizobia in the fallow field was consistent with other previous
214 work by Abaidoo et al. (2007). Higher fertility status of Idi Ayunre soil compared to UITRF
215 soil can also be linked to the fallow and cropping history of the two locations and it reflected
216 in the higher biomass dry weight of lablab in Idi Ayunre soil compared to UITRF soil in the
217 pot experiment.

218 The inoculation of lablab by the indigenous isolated strains yielded more nodules and
219 N fixation compared to where plants were not inoculated. In the pot experiment, it was
220 clearly shown that the introduced indigenous and exotic strains increased nodulation of lablab
221 more than the native indigenous rhizobia. This shows that inoculant strains were highly
222 compatible and were able to establish a functional symbiotic relationship without much
223 interference of soil native rhizobia or any soil microbes. In other words, the inoculated strains

were able to demonstrate their nodulation potential and out-competed the soil native rhizobia for nodule occupancy. The competitive ability of these introduced strains was also displayed in the field experiment at UITRF where introduced indigenous strains like IDC8 and OISA-6E were able to increase the number of nodules by two fold compared to the indigenous rhizobia in the control treatment. The competitive ability of the introduced strains was probably enhanced by the low rhizobial cell in the two soils. It has been reported that the introduced rhizobia are able to perform better than the native one when the population is <10 cell g-1 soil (Brockwell et al 1995; Slattery and Pearce, 2002).

The nodulation of lablab by the three introduced indigenous strains demonstrated a broad specificity of the three strains for certain crops. These strains were either isolated from soybean or cowpea but were able to cross-inoculate with the two crops (Ojo et al 2015) and also with lablab in this study. Lablab, soybean and cowpea belong to the genera Phasoleae (Andrew and Andrew 2017) and all the three have been found to form nodules with bradyrhizobium (Andrew and Andrew 2017; Steenkamp et al. 2008). *Bradyrhizobium lablabi* that nodulate lablab were also isolated in peanut *Arachis hypogaea* (Chang 2011). Symbiosis specificity is complex, involving fine-tuned signal communication between the symbiotic partners, which can occur at multiple phases of the interaction, ranging from initial bacterial attachment and infection to late nodule development associated with nitrogen fixation (Wang et al 2012). The rhizobium species of the three isolates were not determined in this study but it can be concluded that the three isolates had specificity the three crops.

The N derived from the atmosphere by the plant with and without the introduced rhizobia on the field was between 51-56 % which was within the range reported for other legumes (). Although there was no significance difference in the Ndfa (%) through inoculation, the N fixed in the soil was significantly influenced by rhizobial strains. The introduced indigenous rhizobial strains IDC8 and OISA-6E performed better than the resident

indigenous rhizobia. This is an indication that the use of this isolated indigenous bacteria for inoculation of lablab in the derived savanna of Nigeria could provide more N for the soil especially where the residue are returned back to the soil. Biomass dry weight and N uptake was greatly improved by IDC8 compared to the resident native rhizobia. Yield increase were also observed in some legumes inoculated with isolated strains of indigenous rhizobia even in soil with high population of resident indigenous rhizobia (Mostasso et al., 2002, Mrabet et al, 2005, Mulas et al., 2011). The isolated indigenous strain IDC8 performed better than the combination of IRj 2180A and R25B, the exotic strains, in terms of biomass dry weight, nodule formation and N uptake. Of the three indigenous that were tested, it is only IDC8 that was better than the exotic strains. This is an indication that some indigenous rhizobial strains may compete favourably or out-performed some of the exotic strains that are in use for farmers.

Conclusion

The introduced indigenous rhizobial strains **in soils** hads the capability to effectively fixed N in lablab in the derived savanna of Nigeria. The extent of the performance of the indigenous rhizobia can be influenced by the strain type coupled with other plant and soil factors. Screening for effectiveness of more indigenous rhizobia can provide more effective strains than the exotic ones. With better biological N fixation by lablab using effective rhizobial strains such as IDC8, the crop can serve dual purpose of enriching the soil and providing forage for the farmers.

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373 Table 1**.** Soil physical and chemical properties of the experimental locations

Soil properties	Idi Ayunre	UITRF
pH (KCl)	6.55	5.76
Total N (g kg^{-1})	0.23	0.08
Available P ($mgkg^{-1}$)	0.42	0.13
Ca ($cmolkg^{-1}$)	7.87	4.84
Mg (cmol kg^{-1})	2.59	1.65
K ($cmolkg^{-1}$)	0.83	0.85
Na (cmol kg)	0.44	0.43
Fe (mg/kg^{-1})	25.34	26.29
Mn (mg/ kg^{-1})	14.74	12.38
Sand (gkg^{-1})	645.0	812.5
Clay (gkg^{-1})	185.0	100.0
Silt (gkg^{-1})	170.0	87.5
Textural class	Sandy loam	Loamy sand

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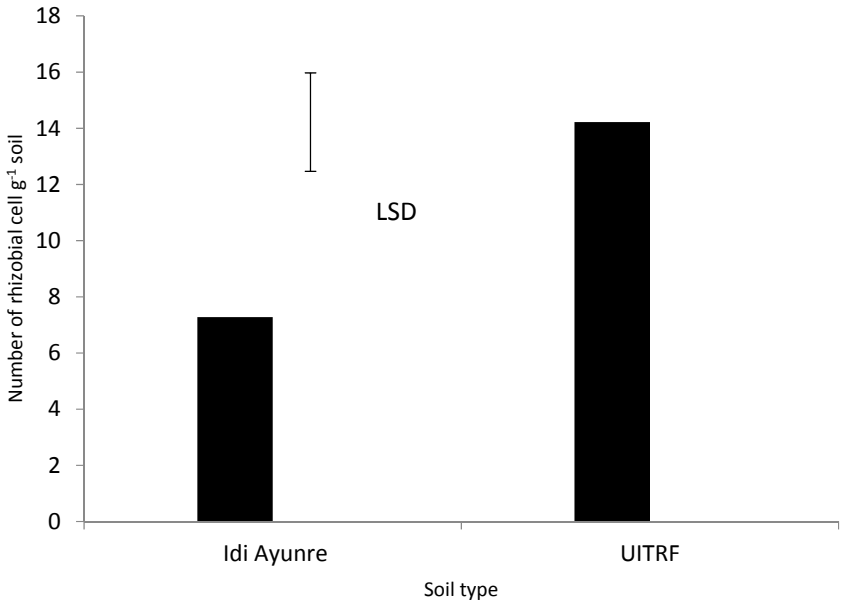

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383 Figure 1: Rhizobial population in the studied locations (Error bar represents LSD at P=0.05)

384 Table 2. Biomass dry weight, nodulation and N derived from atmosphere of lablab as affected

385 by soil type, sterilization and rhizobial inoculation in pot experiment

Treatment		Biomass dry weight (g/pot)	Number of nodules	Nodule dry weight (g/pot)	NDFA (%)
Soil type					
	Idi Ayunre	12.95a	27.28b	2.13a	59.75a
	UITRF	8.77b	40.28a	1.60b	43.57b
	P value	*.0001*	*.0001*	*.004*	*.003*
Sterilization					
	Partially sterile	11.36a	28.69b	1.20b	50.48
	Unsterile	10.36b	38.86a	2.53a	52.84
	P value	*.05*	*.0001*	*.0001*	*.63*
Rhizobial Inoculation					
	OISa-6e	9.88	30.58b	2.08a	49.96

	IDC8	11.78	41.33a	1.97a	52.29
	IRJ 2180A	11.37	37.25ab	1.98a	53.16
	R25	10.72	39.83a	2.02a	54.40
	TRC2	10.03	36.25ab	2.07a	51.39
	Control	11.39	17.42c	1.09b	48.76
	P value	*.16*	*.0001*	*.04*	*.73*

386 Values followed by the same alphabet are not significantly different (P=0.05)

387 Table 3. Interactive effect of soil type, sterilization and rhizobial inoculation on biomass dry

388 weight and nodulation in pot experiment

Soil type	Rhizobial inoculation	Biomass dry weight (g/pot)		Number of nodule		Nodule dry weight g/pot	
		Sterile	Unsterile	Sterile	Unsterile	Sterile	Unsterile
Idi Ayu	OISa-6e	10.58	11.78	20.33	18.00	0.89	4.08
	IDC8	15.20	12.99	36.67	28.33	1.65	2.43
nre	IRj 2180A	15.71	11.94	41.00	29.33	1.89	2.55
	R25	11.22	12.99	12.33	56.00	0.66	4.10
	TRC2	12.73	11.06	16.67	37.00	1.47	3.55
	Control	17.90	11.37	10.00	21.67	0.40	1.94
UITRF	OISa-6e	9.06	8.10	38.67	45.33	1.36	1.98
	IDC8	8.61	10.34	40.00	60.33	1.08	2.71
	IRj 2180A	9.04	8.80	28.67	50.00	1.18	2.32
	R25	10.26	8.40	45.33	45.67	1.57	1.74
	TRC2	7.99	8.34	42.67	48.67	1.42	1.86
	Control	8.06	8.24	12.00	26.00	0.86	1.15
	SE	4.57		8.92		0.34	

389

390 Table 4. Nodulation and N fixation as affected by rhizobial inoculation at UITRF field
391 experiment, Ibadan

Rhizobial inoculation	Number of nodule	Ndfa (%)	N fixed (kg/ha)
OISa-6e	76.0a	54.37	83.6bc
IDC8	76.0a	56.16	119.0a
R25B+IRj2180A	54.7b	56.0	94.3ab
TRC2	50.7bc	51.24	58.8cd
Control	34.0c	55.7	48.7d
P value	*.001*	*.36*	*.001*

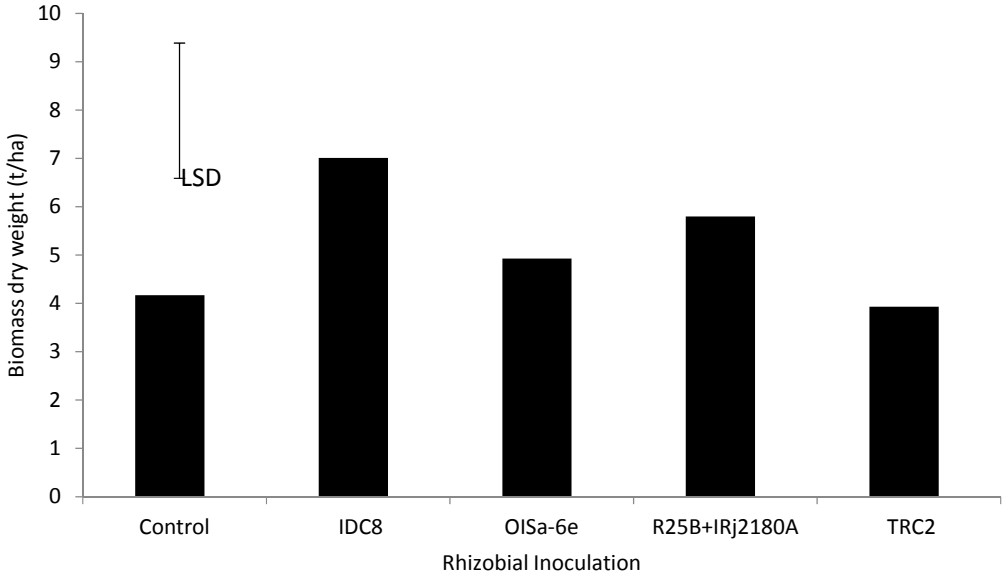
392 Means within column followed by the same alphabet are not significantly different (P=0.05)

393 Table 5. Nitrogen and P uptake and content as affected by rhizobial inoculation at UITRF
394 field experiment

Rhizobial inoculation	N content (% dm[1])	P content (% dm)	N uptake (kg/ha)	P uptake (kg/ha)
OISa-6e	3.12	0.37	153.1ab	18.5
IDC8	3.09	0.38	216.0a	26.1
R25B	2.53	0.28	159.9ab	17.6
TRC2	2.88	0.42	110.3b	17.3
Control	3.02	0.29	104.0b	12.9
P value	*.14*	*.09*	*.05*	*.18*

395 Means within colums followed by the same alphabet are not significantly different (P=0.05);
396 [1] dry matter;
397
398
399
400

401



402

403 **Figure 2:** Biomass dry weights of lablab treated with different strains of rhizobium (Error bar

404 represents LSD at P=0.05).