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Vol. 10(7), pp. 136-144, July 2016
DOI: 10.5897/AJPS2016.1406
Article Number: 511AA4E59268
ISSN 1996-0824
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http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

Response of Sesbania (*Sesbania sesban* L. Merr.) to inoculation with indigenous isolates of Rhizobium strains

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Received 2 March, 2016; Accepted 5 May, 2016

Nitrogen fixation through legume-rhizobium symbiosis serves as a cost effective, sustainable and eco-friendly source of N to fodder and grain legume crops. However, there is a need to identify effective rhizobial inoculants compatible with a particular legume. An experiment was conducted to evaluate the effectiveness of forty indigenous isolates of Rhizobium strains on *Sesbania sesban* L. Merr. Each strain was cultured in yeast manitol broth for 3-5 days and inoculated to sesbania seedlings. Unfertilized (-N) and N fertilized (+N) treatments were also included as control treatments. Results revealed that Rhizobium strains have significantly affected nodulation, growth and N content (NC) of sesbania. Based on their relative effectiveness on seedling growth of sesbania, the test strains were grouped into six clusters. Eight strains (20%) in clusters VI, V and VI produced significantly higher nodulation, growth and NC on seedlings of sesbania than those produced by all other strains and +N treatment. On average, these strains increased shoot dry matter and NC by 50 and 50.8 % over +N treatments, respectively. Their mean symbiotic effectiveness (SE) values were > 85 % and hence are classified as highly efficient strains. In conclusion, there is a significant possibility of being able to isolate effective strains, which can be used as inoculants for sesbania, from rhizobial biodiversity resources in Ethiopian soils.

Keywords: Nitrogen, Rhizobia, green manure, Sesbania.

INTRODUCTION

Almost all Ethiopian soils are by far most deficient in Nitrogen (N), severely limiting crop production and productivity (Bekere et al., 2014). Inadequate uses of organic and inorganic fertilizers, continuous cropping, soil erosion and decreasing or abandoning of traditional soil fertility restoration practices are some of the causes that account for its deficiency (Haile and Abay, 2013).

In an effort to overcome the problem, application of fertilizers containing N has long been practiced in Ethiopia. Dramatic increases in the yield of several crops

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have been obtained due to this practice. There were several occasions where the yields of crops were increased by over 100% (Gebre, 2007). Due to this fact, the adoption of N fertilizers by farmers in Ethiopia was far higher than any other agricultural technology. Consequently, the importation and consumption of the fertilizer increased year on year (Haile and Tekalign, 2013). However, the per capita fertilizer consumption is still very low and farmers are applying sub-optimal levels of fertilizers (IFPRI, 2010). The unprecedented increase in the price of fertilizers is the major challenge for low levels, or a lack of, fertilizer use by farmers. Moreover, continuous use of N fertilizers on the same piece of land acidifies soil and aggravate cation losses and pollution (Rowell, 1994; Haile and Abay, 2013).

These problems necessitate the development and use of alternative sources of N. One such alternative is the use of biological nitrogen fixation (BNF), a process in which atmospheric N is converted into a plant available form of N by free living and symbiotic N_2 fixing microorganism. BNF is a cost effective, sustainable and eco-friendly means of supplying N to plants (Hungria and Vargas, 2000). The scientific exploitation of BNF can greatly decrease our dependence on artificially produced commercial N fertilizer and improve the quality and quantity of internal resources (Arujo et al., 2012).

The use of leguminous green manure crops and trees is one of the mechanisms of taking advantage of BNF. Legumes belonging to the family of Fabaceae form symbiotic association in their roots with a group of microorganisms which belong to the genus Rhizobium. In the process of association the roots of legumes and Rhizobia form a structure known as a nodule in which atmospheric N_2 fixed and converted into plant useable form them and for none N_2 fixing crop (Peoples and Craswell, 1992). Sesbania (*Sesbania sesban* (L) Merr.) is one of the most important and widely used green manure and improved fallow species to replenish N depleted soils by farmers in eastern and southern Africa (Makatiani and Odee, 2007). It is a fast growing, leguminous N_2-fixing, multi-purpose tree adapted to subtropical and tropical environments (Makatiani and Odee, 2007). When sesbania biomass is incorporated into the soil as green manure, it decomposes fast and release substantial amounts of crop available N and organic carbon into the soil (Nigussie and Alemayehu, 2013). For instance, Weerakoon (1989) reported that green biomass of *Sesbania sesban* applied at 4.4 tha^{-1} with N equivalence value 83 kg N ha^{-1} increased the grain yield of maize from 1.9 t ha^{-1} in the unfertilized control plot to 3.9 t ha^{-1} in Sri Lanka.

Sesbania was introduced to Ethiopia in the 1970s and thought as the most promising species in the highlands due to its N_2-fixing ability, deep rooting tree with good quality foliage to be used as feed for animals and protein supplement (Mekoya et al., 2009) and for erosion control and soil fertility restoration (Degefu et al., 2011). It can be integrated into the farming system for soil fertility improvement as alley cropping, where its biomass can be pruned and incorporated into the soil, or for the improvement of the fallow system.

The high value of sesbania as indicated above as an organic N fertilizer source for soil fertility improvement and/or as forage is due to its N-fixing ability in symbiotic association with bacteria, commonly known as rhizobia (Wolde-meskel et al., 2004a). Through this association sesbania can fix up to 542 kg N ha^{-1} $year^{-1}$ (Shaheen et al., 2004). Similarly, Degefu et al. (2011) reported that N-fixation by sesbania is in the range of 500 to 600 kg N ha^{-1} $year^{-1}$.

However, the amounts of N fixed by tree legumes, including sesbania among others, depends on the symbiotic effectiveness of rhizobia nodulating the particular legume. This is due to the fact that there are wide variations among legumes in their specificity for a particular rhizobia strain. Soils may contain several types of rhizobia and yet a strain specific and effectively nodulating a particular legume species may be absent or the population of the appropriate strain could be too small for nodulation to occur (Dart et al., 1991). Thus, in such soils there is a need to inoculate leguminous crops and trees with the effective rhizobia strain which is specific to a particular legume species. There are also promiscuous types of rhizobium strains which are able to effectively nodulate different species of leguminous trees and crops. If such strains are obtained, it will be more advantageous than specific strain nodulating and inducing N-fixation in only one species. In any case, developing a specific or/and promiscuous rhizobia strain for enhanced N-fixation by legumes requires isolation and screening such bacteria from diverse agro ecologies and soil types in the laboratory, greenhouse and fields.

Once such effective rhizobium strains are obtained they can be commercially produced and supplied to farmers or any user so that they can be used as inoculants of sesbania. In this regard, Wolde-meskel et al. (2004a) isolated 241 different rhizobium strains from roots of 15 woody species most of them being indigenous to Ethiopia and three leguminous crops. They further reported that they were belonging to genera of *Agrobacterium*, *Bradyrhizobium*, *Mesorthizobium*, *Rhizobium* and *Sinorhizobium* based on bio-chemical and molecular methodologies. However, information on nodulating ability of these strains and their effects on the growth and N content of legumes is lacking. Such information is important to identify effective rhizobium strains that can be used as inoculants of a particular legume which ultimately helps to produce high quality sesbania green manures that will be used to fertilize the soil. Thus, an experiment was conducted to investigate the ability of selected indigenous strains of rhizobia in inducing nodulation in sesbania and to evaluate the effectiveness of each strain on the growth and N content of sesbania in the greenhouse.

Table 1. Indigenous rhizobium strains, their geographical origin, host plant and their genera.

Rhizobium strains codes	Geographical origin (locations in Ethiopia)	Host plant	Genera
AC51a$_1$	Nazret	*S. sesban*	*Rhizobium*
AC51a$_2$	Nazret	*S. sesban*	*Rhizobium*
AC51c	Nazret	*S. sesban*	*Rhizobium*
AC61a	Debrezeit	*V. unguiculata*	*Rhizobium*
AC 61d	Debrezeit	*V. unguiculata*	*Rhizobium*
AC73d	Debrezeit	*P. vulgaris*	*R.huautlense*
AC73b$_1$	Debrezeit	*P. vulgaris*	*Rhizobium*
AC73b$_2$	Debrezeit	*P. vulgaris*	*Rhizobium*
AC73c	Debrezeit	*P. vulgaris*	*Rhizobium*
AC47c	Arba-minch	*S. sesban*	*S.fredii*
AC73e$_2$	Debrezeit	*P. vulgaris*	*Rhizobium*
AC100e	Leku	*A. senegal*	*M. abyssinicae*
AC100c	Leku	*A. senegal*	*M. abyssinicae*
AC28c$_2$	RFC	*A. tortilis*(Meki)	*Mesorhizobium*
AC39a	Chofa	*A. abyssinica*	*M. shonense*
AC39e$_1$	Chofa	*A. abyssinica*	*M. shonense*
AC 39d	Chofa	*A. abyssinica*	*M. shonense*
AC38b$_2$	Akaki	*A. abyssinica*	*Sinorhizobiumi*
AC39e$_2$	Chofa	*A. abyssinica*	*M. shonense*
AC99a	Wondogenet	*S. sesban*	*M. hawassense*
AC99b	Wondogenet	*S. sesban*	*M. hawassense*
AC99c	Wondogenet	*S. sesban*	*M. hawassense*
AC99e	Wondogenet	*S. sesban*	*M. hawassense*
AC98a	Wondogenet	*A. abyssinica*	*M. abyssinicae*
AC98b	Wondogenet	*A. abyssinica*	*M. abyssinicae*
AC98c	Wondogenet	*A. abyssinica*	*M. abyssinicae*
AC98e	Wondogenet	*A. abyssinica*	*M. abyssinicae*
AC40a	Debrezeit	*A. abyssinica*	*Sinorhizobium*
AC21c$_2$	Nazret(A.minch)	*A. tortilis*	*M.plurifarium*
AC51e	Nazret	*S. sesban*	*Rhizobium*
AC50b	Debrezeit	*S. sesban*	*Rhizobium*
AC50c	Debrezeit	*S. sesban*	*Rhizobium*
AC50d	Debrezeit	*S. sesban*	*Rhizobium*
AC18a	Debrezeit	*A. tortilis*(Abergele)	*S.fredii*
AC20b	Akaki	*A. tortilis*(A. minch)	*S.fredii*
AC21c$_1$	Nazret	*A. tortilis*(A. minch)	*Sinorhizobium*
AC25a	RFC	*A. tortilis*(Mega)	*Sinorhizobium*
AC47a	Arba-minch	*S. sesban*	*Sinorhizobium*
AC47b	Arba-minch	*S. sesban*	*Sinorhizobium*
AC46d	Arba-minch	*S. sesban*	*Sinorhizobium*

MATERIALS AND METHODS

Brief description of the study site

The experiment was conducted in Hawassa, southern Ethiopia in the greenhouse of the College of Agriculture, Hawassa University, which is located at 7° 05" N and 38° 47" E in 2013. It has a mean altitude of 1750 m above sea level with mean minimum and maximum temperature of 12.06 and 26.68°C respectively. It receives a mean annual rainfall of 952 mm.

Treatments and experimental procedure

Treatments included 40 Rhizobia strains isolated from roots of different plant species and diverse locations of Ethiopia (Table 1). Moreover, un-inoculated negative (-N) and un-inoculated but N fertilized (+N) control treatments were also included in the experiment for comparison. The rhizobium strains were originally isolated, authenticated and their phylogenetic identities were established by Wolde-meskel et al. (2004 a, b). They were maintained in soil microbiology laboratory, College of Agriculture,

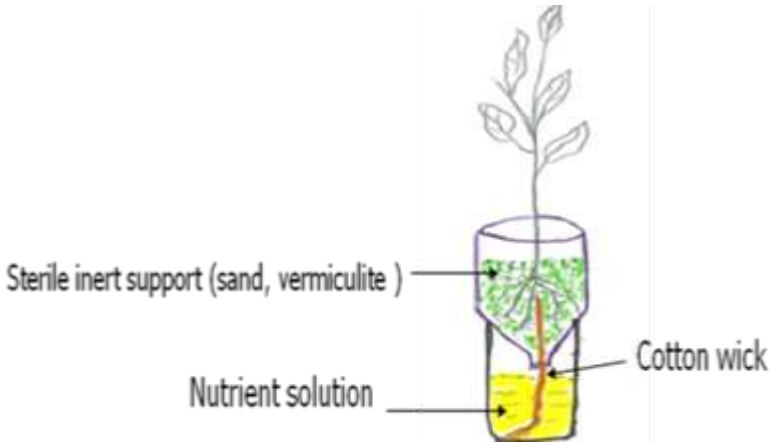

Figure 1. Model of modified Leonard jar containing cotton wick, nutrient solution and sterile inert support.

Hawassa University, Ethiopia and were generously made available by the college for this study. The list of strains along their geographic origin and host plants from which they were first isolated are indicated in Table 1.

The experiment was conducted in the greenhouse using modified Leonard jars, assembled as described in Vincent (1970). The jars were made of two plastic jugs of different sizes. The upper part of the jar had a diameter of 6 cm wide at the mouth and was filled with sterilized sand. The lower part of the jar was used to fill the N free nutrient solution (Figure 1).The cotton wick in the middle makes the nutrient available to the growing plant.

Parallel to preparing the Leonard jars, the laboratory sterilization and pre-germination of sesbania seeds were conducted. Visually healthy looking seeds of sesbania were selected and the surface sterilized with 96% alcohol followed by surface disinfection with 3% sodium hypochlorite for one minute (Somasegaran and Hoben, 1994). Then, the seeds were rinsed with distilled water five times. The disinfected seeds were aseptically transferred into petri-dishes containing 1% water agar medium and allowed to germinate for 12 days at room temperature.

The germinated seeds of sesbania were transplanted to the Leonard jar using sterilized forceps. Two seedlings were planted per jar and later thinned to one seedling per jar. The seedlings in the Leonard jar were fertilized with quarter strength of Modified Jensen N- free solution (Broughton and Dilworth, 1970) twice a week. In the meantime, each strain indicated in Table 1 was grown on yeast extract manitol broth (YMB) for 3 to 5 days, depending on the genera specific of strain. After the emergence of the first leaf (5 days after transplanting), the seedlings of were inoculated with each YMB cultured strains containing approximately 10^9 cell ml^{-1} as per the treatment. Un-inoculated and un-fertilized (-N) control treatment was also supplied with N fertilizer free medium. The un-inoculated but N fertilized (+N) treatment plants were supplied with 1 g of KNO_3 L^{-1} of the nutrient solution (Broughton and Dilworth, 1970). The experiment consisted of a total of 42 treatments and was laid out in completely randomized design (CRD) with three replications. The plants were grown for 9 weeks.

Two weeks after seedlings were inoculated with rhizobium strains, data on plant height (PLHT) and number of leaves (LN) were collected every week until the end of the experiment. The plants were harvested at the end of 9^{th} week. Tops were severed at sand level dried at 70°C in an oven for 48 h and weighed. The roots were carefully separated from the sand from each jar and immediately brought to the nearby soil laboratory where they were washed over 1 mm sieve by gently flowing tap water in order to remove adhering sand and other dust particles. Then nodules were counted. Both the nodules and roots were then weighed after oven drying at 70°C as per the treatment, and the replications were taken.

The dried shoots of each seedling were further processed to demine the total tissue N content. The dried tissues of each seedling were pass through a 0.5mm sized sieve. Then the processed samples were analysed for total N content (NC) by using Kjeldhal digestion procedure as described in Rowell (1994).

Determination of symbiotic efficiency of Rhizobia isolates

Symbiotic efficiency (SE) of each isolate was calculated using the following formula:

$$\text{SE (\%)} \frac{N \text{ in the shoot dry matter of inoculated plant } X\ 100}{N \text{ in the shoot dry matter of N fertilizered plant}}$$

Symbiotic effectiveness (SE) values were rated as ineffective (< 35%), effective (35-85%) and highly effective (>85%) as described in Beck et al. (1993).

Statistical analysis

Data on the number of nodules per plant (NN), nodule size (NS), nodule dry weigh (NDW), shoot dry weight (SDW), root dry weight (RDW), PLHT, NL and tissue N contents (NC) were subjected ANOVA using SAS software version 8.1 (SAS, 2000). Further, mean separations were done using least significant difference method at 0.05 probability level. Cluster analysis of the rhizobium strains studied in experiment was performed using the same software based on nodulation, growth parameters and the NC of sesbania (functional diversity).

RESULTS AND DISCUSSION

Functional diversity of test strains

All strains induced nodulation in sesbania seedlings except AC100e and the results of cluster analysis

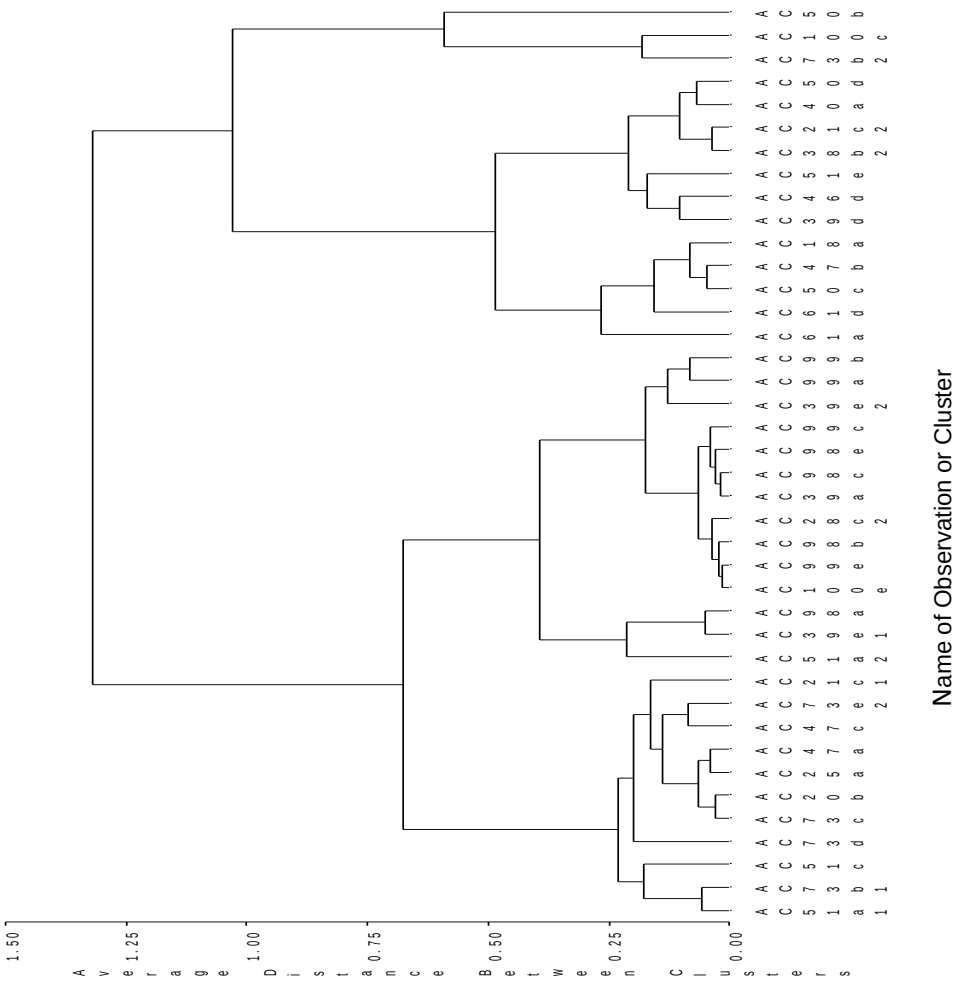

Figure 2. Dendrogam showing the functional relationship or relationships among rhizobium strains nodulating sesbania.

revealed that all the forty rhizobium strains were classified into six clusters. The dendrogram of the six cluster developed based on the functional relationship of the strains in each of the six cluster is shown in Figure 2 and the list of rhizobium strains in each cluster and their proportion are presented in Table 2. The strains in cluster-I account for the largest proportion (35%) compared with all other clusters, followed by cluster-II (27.5%), and the least proportion (2.5%) of strains were found in cluster-VI.

According to Wolde-meskel et al. (2004b, c), AC61a AC61d and AC73b2 were found to be genetically highly related based on RFLP fingerprinting. Similarly, the same authors found that AC50, AC50c and AC50d were genetically and metabolically in the same group. However, the dendrogram developed based on functional diversity in this study has grouped test strains in different clusters (Figure 2). This shows that strains of similar genetic and physiological origin do not necessarily have similar symbiotic effectiveness.

Cluster mean effects of strains on nodulation and growth of sesbania

The mean nodulation and plant growth parameters, tissue N content in sesbania produced by rhizobium strains in each cluster and their mean symbiotic effectiveness values (SE) are presented in Table 3. Strains in cluster-I and II accounted for 62.5% of all the test strains and produced lower growth parameters and tissue N content in sesbania than that produced in N fertilized (+N) treatment. Furthermore, their mean SE values were less than 35% and thus, they were classified as symbiotically ineffective (Beck et al., 1993). Therefore, strains in these clusters were inferior to N fertilizer treatment based on all indices of symbiotic efficiency. On the other hand, the strains in cluster III produced growth parameters and N content in sesbania similar to that produced in N fertilized treatment. Their mean SE value was 101% which is greater than 85% indicating that they are symbiotically effective and have a potential to be

Table 2. List of rhizobium strains in each cluster and their proportions.

Clusters	List/codes of rhizobium strains	Number of strains in each cluster	Proportion (%)
I	AC100e, AC28c$_2$, AC39a, AC39e$_1$, AC39e$_2$, AC99a, AC99b, AC99c, AC99e, AC98a, AC98b, AC98c, AC98e, AC51a2	14	35
II	AC73c, AC51a1, AC20b, AC25a, AC51a$_1$, AC73b1, AC47c, AC73e2, C21c, Ac51c,AC73d	11	27.5
III	AC40a, AC21c$_2$, AC38b2, AC51e,AC50d, AC39d, AC46d	7	17.5
IV	AC50c, AC47b, AC18a, AC61d, AC61a	5	12.5
V	AC100c, AC73b2	2	5
VI	AC50b	1	2.5
Total	**-**	**40**	**100**

Table 3. Cluster mean effects of rhizobium strains on nodulation, growth and N content of sesbania and their symbiotic efficiency (SE) of Rhizobia isolates.

Rhizobia clusters	*NN	NS (cm)	NDW (mg plant^{-1})	SDW g plant $^{-1}$	RDW g plant $^{-1}$	PLHT (cm)	NL	N (mg plant^{-1})	SE (%)
Cluster-I	12	0.7	25	0.20	0.1	18	7.0	2.5	25.8
Cluster-II	43	1.9	62	0.7	0.3	32	11.2	3.3	34
Cluster-III	44	1.9	66	0.8	0.5	36	11.6	9.8	101
Cluster-IV	62	2.2	82	1.0	0.6	38	11.5	12.0	124
Cluster-V	68	2.7	115	1.2	0.7	41	13.8	14.3	147
Cluster-VI	76	2.8	130	1.4	1.0	51	12.7	19.6	202
-N control	0	0	0	0.11	0.08	12	4.7	1.2	-
+N control	0	0	0	0.8	0.46	31	10.3	9.7	-

*NN = number of nodulest, NS = nodule size, NDW = nodule, dry weight, SDW = shoot dry weight, RDW = root dry weight, PLHT = plant height, LN = leaf number, N = Nitrogen and SE = Symbiotic Efficiency.

exploited as inoculants of sesbania.

However, those strains in clusters IV, V and VI produced a superior mean growth and N contents than that produced in +N treatment. This finding has also been substantiated by their very high SE values (Table 3). The strains in these clusters accounted for only 20% of all strains screened.

Effects of individual strains in clusters from III-VI on sesbania seedlings

The effect of individual rhizobium strains in cluster III, IV, V and VI on the nodulation, growth and N content of sesbania are summarized in Table 4 and 5. Accordingly, there is a significant variation in the strains in relation to the nodulation and growth of sesbania. The highest NN, NS, NDW and SE were recorded in sesbania seedlings inoculated with AC50b followed by AC100c, AC73b2 and AC50c in that order, while AC39d and AC50d were the lowest (Table 4).

There is a significant variation in the Rhizobium strains in relation to the nodulation and growth of sesbania. Eight (20%) of all tested strains produced significantly higher shoot and root dry matter yields and higher plant height and tissue N content in sesbania seedlings than that produced in the N fertilized treatment. However, the highest growth and N content in sesbania seedlings were still obtained from inoculants of AC50b, AC100c and AC73b2. These inoculants for example increased SDW by 64, 39 and 40% over that obtained with N fertilizer treatment respectively. The corresponding increases in PLHT were 64, 36 and 29 % respectively. On the other hand, these inoculants increased the N contents of sesbania seedlings by 102, 41 and 53.6% over +N treatment respectively. This is in line with the findings of Makatiani and Odee (2007) who reported that inoculation of sesbania with indigenous rhizobia isolate GSS1 significantly increased SDW and NC by 23 and 20% respectively over the control in the pot experiment. The apparent superior growth and N contents observed in sesbania seedlings which were inoculated with these three strains could be due to their higher N input to the plant through N_2 fixation. Figure 3 shows the dramatic (left) effect of AC50b on sesbania seedling relative to the negative control (middle) and positive control (right)

Table 4. The effects of rhizobium strains on nodulation parameters of sesbania and their SE.

Treatment	NN	NS (cm)	NDW (mg plant^{-1})	SE (%)
-N control	0h†	0^{f}	0g	-
AC61a	74.0^{a}	2.6^{a}	76.7^{de}	127
AC61d	71.0^{a}	2.2^{bc}	95.0^{cd}	133
AC73b2	70.0^{a}	2.6^{ab}	107.5^{bc}	154
AC100c	73.3^{a}	2.7^{a}	116.2^{ab}	141
AC39d	39.3^{f}g	2.3^{bc}	55.1^{f}	109
AC38b2	49.3^{cde}	2.0^{de}	79.5^{de}	106
AC40a	34.0g	0.23^{f}	57.3^{f}	98
AC21c2	46.3^{def}	2.4^{bc}	72.0^{f}	98
AC51e	61.3^{b}	2.2^{cd}	73.4^{f}	106
AC50b	76.0^{a}	2.8^{a}	125.8^{a}	202
AC50c	56.7^{bc}	1.8^{e}	71.0^{ef}	120
AC50d	39.3^{f}g	2.2^{cd}	62.2^{f}	95
AC18a	57.0^{bc}	2.8^{a}	85.4^{de}	121
AC47b	52.0^{cd}	2.7^{a}	79.1^{de}	119
AC46d	43.7^{ef}	2.1^{ef}	57.8^{f}	97
+N control	0h	0^{f}	0g	-
LSD 0.05)	**7.9**	**0.33**	**18.3**	**-**
CV (%)	**9.5**	**10.2**	**15.4**	**-**

†Means within column followed by the same letter (s) are not statistically different from each other at 0.05 probability level.

Table 5. Effects of rhizobium strains on growth and N content of Sesbania 9 weeks after planting.

Treatments	SDW (g plant^{-1})	RDW (g plant^{-1})	PLHT (cm)	NL	NC (mg plant^{-1})
-N control	0.08^{f}	0.034^{g}			
AC61a	0.91^{de}	0.18^{cde}	35.0^{d}	10.7^{d}	12.3^{cde}
AC61d	1.02^{bc}	0.18^{cde}	39^{bcd}	11.0^{bc}	12.9^{cd}
AC73b2	1.15^{b}	0.22^{bc}	40.0^{bc}	13.0^{ab}	14.9^{b}
AC100c	1.15^{b}	0.25^{ab}	42.3^{b}	14.3^{a}	13.7.^{bc}
AC39d	0.9^{cde}	0.14^{ef}	39.3 ^{cd}	12.0^{bcd}	10.6^{efg}
AC38b2	0.86^{cde}	0.12^{ef}	37.3^{cd}	11.3^{cd}	10.3^{fg}
AC40a	0.81^{e}	0.18^{cde}	32.0^{f}	12.0^{bcd}	9.5^{g}
AC21c2	0.81^{e}	0.17^{de}	35.0^{ed}	12.3^{bcd}	9.5^{g}
AC51e	0.88^{cde}	0.16^{ef}	38.5^{cd}	13.7^{ab}	10.3^{fg}
AC50b	1.35^{a}	0.3^{a}	51.0^{a}	13.0^{ab}	19.6^{a}
AC50c	1.0^{bcd}	0.22^{bcd}	40.3^{bc}	12.0^{bcd}	11.6^{def}
AC50d	0.77^{e}	0.14^{ef}	36.7^{cd}	10.7^{d}	9.2^{g}
AC18a	0.95^{cde}	0.14^{ef}	38.7^{bcd}	12.0^{bcd}	11.7^{def}
AC47b	1.0^{bcd}	0.14^{ef}	37.0^{cd}	12.0^{bcd}	11.5^{def}
AC46d	0.84^{de}	0.13^{ef}	35.0^{de}	11.3^{cd}	9.4^{g}
+N control	0.83^{ed}	0.14^{ef}	31.^{f}	10.3^{d}	9.7^{g}
LSD 0.05)	**0.18**	**0.05**	**3.7**	**1.85**	**1.74**
CV (%)	**12.5**	**18**	**6.1**	**9.7**	**9.5**

Means within column followed by the same letter (s) are not statistically different from each other at 0.05 probability level.

treatments.

The majority of strains in Table 4 or 5 which were found to be effective inoculants of sesbania were found to belong to the genera of *Rhizobium* and *Sinorhizobia*. The

Figure 3. Sesbania seedlings as they were grown in Leonard Jars in the greenhouse. Seedling inoculated with AC50b (Left), seedling without N fertilizer and without inoculants (Middle), and seedling received N fertilizer (Right).

test strains in the two genera accounted for 53.3 and 33.3% of all the test strains. This is in line with Wolde-meskel et al., (2004a) who reported that most strains that produced higher plant height and dry matter in sesbania belonged to the genera of *Rhizobia* and *Sinorhizobia*. The apparent large number of N_2 fixing bacteria which we found to be effective in nodulating and enhancing the growth and N content of sesbania suggests that the tree is promiscuous both for nodulation and effectiveness. Similarly, Boivin et al. (1997) reported that sesbania species can enter into symbiosis with many other species of rhizobia.

Conclusion

It is concluded that out of the forty rhizobium strains screened for their symbiotic effectiveness in the greenhouse, 37.5% of them produced dry matter yield, plant height and tissue N content in sesbania similar or higher than that produced in +N fertilizer treatment. Of this number only eight (20%) of them produced significantly higher dry matter, plant height and tissue N content in sesbania than that produced in +N control treatment. This shows that there is a high potential to identify effective rhizobium strains from indigenous sources from the diverse agro ecologies of Ethiopia. This, further implies that some of the efficient strains identified in this study can be used as inoculants of sesbania for enhanced production and productivity of the tree which in turn can be exploited as green manure or/and forage. Most of the rhizobium strains identified as effective symbionts of sesbania were originally isolated from trees and low land food leguminous crops other than sesbania. This implies that there is possibility to use one or more of the rhizobium strains identified as efficient in this study as inoculants of many legumes.

Conflict of Interest

The authors have not declared conflict of interest.

ACKNOWLDGEMENT

We would like to thank the legume Rhizobium symbiosis research project (NUFU) funded by the Norwegian government for financing this study. The technical support from N2Africa-Ethiopia is well appreciated.

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