https://doi.org/10.46344/JBINO.2022.v11i03.24

EVALUATION OF RHIZOBIAL STRAINS FOR SWEET LUPINE GROWN IN ACID-PRONE AREAS OF AWI ZONE BANJA DISTRICT OF ETHIOPIA

***Wubayehu Gebremedhin[1], Mamo Bekele[2], Getachew Yilma[3] and Mesfin Kuma[4]**

1, 3 Ethiopian Institute of Agricultural Research, Fogera National Rice Research and training Center, P.O. Box: 1937 Woreta, Ethiopia
2 Ethiopian Institute of Agricultural Research, Holeta National Biotechnology Research Center, Holeta, Ethiopia
4 Ethiopian Institute of Agricultural Research, Pawe Agricultural Research Center, Pawe, Ethiopia

Email: - wubsee6@gmail.com

ABSTRACT

Earlier research, has demonstrated the existence if specificity between hosts white lupin (*Lupinus albus* L.) plant and *Bradyrhizobium* strain and also it has been stated that Lupins, like many other species belonging to the *Leguminosae*, are able to initiate a symbiotic relationship with bacteria of the family *Rhizobiaceae*. Despite the interest of this symbiosis there are few studies about the identity of strains nodulating lupins and there is a lack of precise information on the evaluation of native *bradyrhizobia*-sweet lupine interaction for better agronomic performance of the crop. Therefore, this project focuses on evaluation of our renewable nitrogen fixing resources in agriculture sector particularly for sweet lupine production on two selected cultivars with the objective of evaluating the productivity of test cultivars with and without the presence of the two test rhizobial inoculants under acid prone areas. A two season experiment was conducted in RCBD design of two varieties in factorial combination with two rhizobial strains as well as the positive and negative control treatments. The outcome of this experiment with non significant difference not only among the test inoculants but also between control treatments showed the growth and productivity of both sweet lupine varieties were not affected due to the presence or absence of tested bio fertilizers. This might be an indicator either the presence of competitive native micro flora indicating the poor performance of test strains or the test cultivars *SW-001* and *Vitabor* might have exceptional potential for accessing their nutritional demand. In the study sites both *Vitabor* and *SW-001* lupine cultivars has been grown and gave nearly equivalent agronomic yield. Therefore, this might encourage further works in looking for best native competitive rhizobial strain to be investigated for such varieties.

Keywords: *Cultivars; Symbiosis; Rhizobial inoculants*

Introduction

Lupines are legumes which have been cultivated in Europe for the last 2000 years, used in human and animal feeding, as green manure in agriculture (Rosolem *et al*., 2002; Jensen *et al*., 2004) and in soil stabilization. This plant is currently considered a good alternative as an animal foodstuff due to the high quality of its proteins (Erbas *et al*., 2005; Faligowska *et al*., 2007). As the FAO lists grain legumes only, and not forage, fodder and a substantial portion of the world's supply of organic nitrogen is fixed via the symbiosis between root nodulating rhizobial bacteria and leguminous host plants (Postgate, 1998).

Earlier research, has demonstrated the existence if specificity between hosts white lupin (*Lupinus albus* L.) plant and Bradyrhizobium strain (Robinson *et al*., 2000). Additional studies have indicated that white lupin has tremendous potential and can be successfully used as a legume cover crop to support production of summer crops such as sweet corn and muskmelon (Bhardwaj, 2006). Currently there is a lack of precise information on the evaluation of native bradyrhizobia-sweet lupine interaction for better agronomic performance of the crop.

The most important N2-fixing agents in agricultural systems are the symbiotic associations between crop and forage/fodder legumes and rhizobia. With this association each year, about 175 million ton of N is contributed by BNF globally (Burns and Hardy, 1975), of which nearly 79% is accounted for by terrestrial fixation. A near-term strategy for increased fixed-N input to legumes involves a better match of rhizobial Microsymbiont to its host cultivar, earlier initiation and prolongation of symbiotic fixation.

Lupins, like many other species belonging to the Leguminosae, are able to initiate a symbiotic relationship with bacteria of the family *Rhizobiaceae*. Despite the interest of this symbiosis there are few studies about the identity of strains nodulating lupins (Barrera *et al*., 1997; Stepkowski *et al*., 2005; Andam, Parker, 2007).

In context of both the cost and environmental impact of chemical fertilizers, excessive reliance on the chemical fertilizers is not viable strategy in the long run because of the cost, both in domestic resources and foreign exchange, involved in setting up of fertilizer plants and sustaining the production. In this context, biofertilizers would be the viable complementary option for the livelihood of farmers and the environment.

Inoculation of seeds or soil with nitrogen fixing microorganisms increases the microbial population in the rhizosphere, consequently affecting the plant growth. Providing nitrogen through nitrogen fixation by *Rhizobium*, *Bradyrhizobium*, *Azotobacter*, *Azospirillum* have given good results in experiments carried out in Ethiopia and elsewhere. Currently, the N-fixing biofertilizers are the most out reached biofertilizer products to small holder farmers in the country. Practically, there is no an alternate N-fixing biofertilizer type for a given legume type. However, the country has the potential to produce

many N-fixing commercial biofertilizer products. Therefore, this project focuses on evaluation of our renewable nitrogen fixing resources in agriculture sector particularly for sweet lupine production on two selected cultivars. Moreover, the project actively focuses on introduction and screening of exotic N-fixing as well as nutrient solubilizing organisms. With this it has been enabled to evaluate the performance of sweet lupine cultivars productivity under acid prone areas and to evaluate developed rhizobial biofertilizer for lupine in acid prone areas.

Materials and methods

The field experiments were conducted during 2016/17 and 2017/18 main cropping seasons for experiment one and two, respectively.

The treatment structure of this experiment factorial including sweet lupin variety factor having levels of *SW-001* and *Vitabor* and lupin rhizobial isolate factor having levels of *Lup-AH11*, *Lup-A14*, non-inoculated and 18kg N/ha applied from urea. The treatments was replicated three times and laid in RCBD in factorial arrangement. Seed rate is 80kg/ha and plant spacing is 40cm by 10cm where the maximum plot size 3m by 4.2m . Phosphorus was applied uniformly to all plots at a rate of 46kg P_2O_5 per ha in the form of TSP. All agronomic and management operations is practiced in uniform manner.

Data to be collected

The distance between rows and plants are 40cm are 10cm. respectively. The distance between two plots and replication was 1m and 1.5m respectively. The net plot area for each plots are 3mX2.8m=8.4m^2. There are seven rows, hence; four harvestable rows, one disturbed sample row and two border rows. 46 kg/ha P_2O_5 was applied as basal to all treatment as constant variable. Nodulation data: - nodule number and nodule dry weight as well as yield and yield related data were taken. All measured soil and plant data were subjected to ANOVA and mean separation.

Treatment arrangement

S.N	Treatment
1	SW-001 only
2	SW-001+18N/ha
3	SW-001+Lup AH11
4	SW-001+Lup A14
5	Vitabor only
6	Vitabor +18N/ha
7	Vitabor +Lup AH11
8	Vitabor +Lup A14

Results

Table 1. Lupine strain evaluation Banja on farm Site 1 year 1

Varieties	PH cm	BPP No	PPP No	BMY Kg ha^{-1}	GY Kg ha^{-1}	HSW gm
SW-001	71.50b	4.87	28.43	4216.27	1882.88	13.15b
Vitabor	81.97a	4.58	30.95	4146.83	1997.22	14.50a
LSD 0.05	3.878	ns	ns	ns	ns	0.4472
Strains						
no input (control)	79.93	4.47	26.67	3650.79	1754.26	14.60
Lup AH11	78.53	4.67	29.87	4126.98	2014.20	14.73
Lup A14	84.13	4.73	32.07	4404.76	2067.98	14.20
18N kg/ha	85.27	4.47	35.20	4404.76	2152.41	14.47
LSD 0.05	ns	Ns	ns	ns	ns	ns
CV %	5.8	18.6	29.7	22.5	16.2	4

PH= plant height, BPP= branch per plant, PPP= pod per plant, BMY= biomass yield, GY= grain yield, HSW= hundred seed weight

Table 2. Lupine strain evaluation Banja on farm Site2 year 1.

Varieties	PH cm	BPP No	PPP No	BMY Kg ha^{-1}	GY Kg ha^{-1}	HSW gm
SW-001	62.48b	8.65	31.88a	**3779.76a**	**1636.42a**	14.10
Vitabor	70.77a	9.92	17.93b	2500.00b	744.33b	14.47
LSD 0.05	6.166	ns	6.489	742.8	391.4	ns
Strains						
no input (control)	66.97	8.30	21.13	2837.30	980.99	14.53
Lup AH11	65.40	9.33	27.50	2976.19	1169.22	14.40
Lup A14	65.67	9.57	25.33	3432.54	1398.44	14.27
18N kg/ha	68.47	9.93	25.67	3313.49	1212.86	13.93
LSD 0.05	ns	ns	ns	ns	ns	ns
CV %	10.3	21.6	31.9	28.3	39.5	5.9

PH= plant height, BPP= branch per plant, PPP= pod per plant, BMY= biomass yield, GY= grain yield, HSW= hundred seed weight

Table 3. Lupine strain evaluation Banja on farm Site1 year 2

Varieties	PH /cm	BMY (Kg /ha^{-1})	GY (Kg /ha^{-1})	ThSWt (gm)
Vitabor	75.58a	8326.4a	2454a	13.62b
SW-001	85.78b	6618.1b	1987.6b	15.38a
LSD 0.05	3.9514	841.59	278.64	0.8949
Strains				
no input (control)	76.7b	7750ab	2252.2a	14.78a
Lup AH11	81.53ab	8055.6a	2482.9a	14.75a
Lup A14	84.17a	7472.2ab	1848.5b	14.36a
18N kg/ha	80.33ab	6611.1b	2300a	14.1a
LSD 0.05	5.5881	1190.2	394.05	ns
CV %	5.6	12.8	14.3	7

PH= plant height, BMY= biomass yield, GY= grain yield, ThSWt= Thousand seed weight

Table 4. Lupine strain evaluation Banja on farm two years combined analysis result

Varieties	PH cm	BMY Kg ha^{-1}	GY Kg ha^{-1}	ThSWt gm
Vitabor	76.1a	4326.4a	1456.19a	13.94a
SW-001	73.26b	4071.8a	1479.05a	14.2a
LSD 0.05	2.6976	ns	ns	ns
Strains				
no input (control)	73.48a	4412a	1389.9a	13.86a
Lup AH11	73.84a	4412a	1573a	14.37a
Lup A14	76.48a	4333.3a	1408.3a	14.1a
18N kg/ha	75.29a	3958.3a	1499.3a	13.97a
LSD 0.05	ns	ns	ns	ns
CV %	7.6	18.9	21.1	9.6

PH= plant height, BMY= biomass yield, GY= grain yield, ThSWt= Thousand seed weight

In many of tested parameters over the two years experiment significant variation were not observed among the treatments. During the second season experiment the significant variation on agronomic yield was observed across the varieties and strains and *Vitabor* and *Lup AH11* were the maximum yielder variety and strain respectively. But since many of tested parameters during the first season experiments were not significantly different and the cumulative summary (**Table 4**) indicated that many of tested parameters were not significantly affected with strain and variety.

In this experiment the plant base and yield data were analyzed. In the analysis of the second season (2018GC) achievements, at farmer site (**Table 3**), significant differences on all tested parameters were observed. These variations were due both varietal and inoculants application difference. In all tested parameters rhizobial inoculants called *Lup AH11* gave the highest mean yield. Whereas *Vitabor* variety gave the highest mean yield as compared to other N fertilizers sources and test variety respectively.

On the other hand in all location of the first season trials as well as the two years combined results showed significant variations were not observed in all measured parameters among the nitrogen fertilizers and between tested two cultivars. The outcome of this experiment with non significant difference not only among the test inoculants but also between control treatments showed the growth and productivity of both sweet lupine varieties were not affected due to the presence or absence of tested bio fertilizers. Additionally it become a sign of the presence of native rhizobial contributing better growth for both sweet lupine varieties in the study sites of first season experiment. This encourages further investigation of the molecular or genetic performance on nitrogen fixing behavior of sweet lupines.

Sweet lupine variety called *SW-001* showed dominant performance in its mean yield in the second season whereas in the second season *Vitabor* gave dominant performance and non significant difference were observed on the two years combined analysis results. These non significant performance of tested rhizobial strains will remind to reinitiate further work of looking for best native rhizobial strain development from the sites where dominant mean yield obtained from the negative control treatments.

Discussion

The non significant result among all nitrogen fertilizer sources in lines with the previous study output of Fernández P *et al*., 2007 who stated lupine that has a legume of great agronomic potential due to its optimistic effect on soil fertility enhancement naturally. This is mainly due to having unique nodule called lupinoid nodules. These nodule structures have special symbiotic operational mechanism which enables the plant to be resistant to a biotic stresses. According to Michin *et al*., 1992 this might be due to the presence of

the oxygen barrier which have slow response for stress conditions as compared to other legumes. Therefore, this genetic nature of the crop enables to give a yield nearly equivalent to the fertilized and inoculated treatments.

However, the previous study by Fernández P *et al*., 2007 and (Karim A. *et al*., 2012) showed that *Bradyrhizobia-lupine* symbiosis with effective inoculants enhances the stresses tolerance of soil acidity, salinity, and heavy metal toxicity our tested rhizobial strains were not showed special performance as compared to the negative control or fertilized treatment. This might indicates the poor performance of test strains.

Lupine cultivars *SW-001* and *Vitabor* were not affected in their agronomic yield due to the application of test strains. However, previously no one reported host specific strain interaction on sweet lupine (Staples K. *et al*., 2017) were reported the utilization of rhizobial starin will not improve the alkaloid content of particular lupine cultivar.

Conclusion

Rhizobial strains *Lup AH11* and *Lup A14* were not significantly affected the agronomic yield of both sweet lupine cultivars as compared to the negative control and nitrogen fertilized treatments. In the study sites both *Vitabor* and *SW-001* lupine cultivars has been grown and gave nearly equivalent agronomic yield. The equivalent performance of negative control treatment with both inoculated and N fertilized treatments might be an indicator for the presence of host specific native nitrogen fixing micro flora inside the soil therefore, further works in looking for best native competitive rhizobial strain should be investigated.

Acknowledgement

Authors deeply acknowledge the Ethiopian Institute of Agricultural research for the financial support of this work. Also Holeta Agricultural Research center is highly acknowledged for their contribution to supply the lupine seeds and inoculants. Lastly we are pleased to express our gratitude for Pawe Agricultural Research Center gave us all logistic supports for the success of this work.

References

Andam C.P., Parker M.A., 2007. Novel alphaproteobacterial root nodule symbiont associated with Lupinus texensis. Appl. Environ. Microbiol., 73: 5687-5691.

Barrera L.L., Trujillo M.E., Goodfellow M., Garcia F.J., Hernandez- Lucas I., Davila G., van Berkum P., Martinez- Romero E., 1997. Biodiversity of bradyrhizobia nodulating Lupinus spp. Int. J. Syst. Bacteriol., 47: 1086-1091.

Burns RC, Hardy RWF (1975) Nitrogen fixation in bacteria and higher plants. Springer-Verlag, Berlin.

Erbas M., Certel M., Uslu M.K., 2005. Some chemical properties of white lupin seeds (Lupinus albus L.). Food Chem., 89:341-345.

Faligowska A., Szukała J., 2007. Yielding and feeding quality of three lupin species cultivated for silage.**In**: EVALUATION OF BRADYRHIZOBIUM STRAINS FOR LUPINS IN FINNISH CONDITIONS (Karim, A., & Yliopisto,

H., (2012)eds.). Zesz. Probl. Post. Nauk Rol., 522: 229-237. (in Polish).

Fernández-Pascual, José J. Pueyo, María R., de Felipe•M., Pilar Golvano and M. Mercedes Luca (2007). Singular Features of the Bradyrhizobium-Lupinus Symbiosis. *Global Science Books*. Pp.1-12.

Jensen C.R., Joernsgaard B., Andersen M.N., Christiansen J.L., Mogensen V.O., Friis P., Petersen C.T., 2004. The effect of lupins as compared with peas and oats on the yield of the subsequent winter barley crop. Eur. J. Agron., 20: 405-418.

Karim, A., & Yliopisto, H., (2012). EVALUATION OF BRADYRHIZOBIUM STRAINS FOR LUPINS IN FINNISH CONDITIONS University of Helsinki Department of Agricultural Science Plant Production Science / Crop Science Agricultural Science Master degree, (May).

Minchin FR , Ianneta PPM, Fernández-Pascual M, de Lorenzo C, Witty JF, Sprent JI (1992) A new procedure for calculation of oxygen diffusion resistance in legume nodules from flow-through gas analysis data. *Annals of Botany* 70, 283-289 Plants, Springer-Verlag, New York, pp 4-60.

Postgate J., 1998. Nitrogen fixation. Cambridge University Press, Cambridge, UK.

Robinson KO, Beyene DA, van Berkum P, Knight-Mason R, Bhardwaj HL (2000) Variability in plant-microbe interaction between Lupinus lines and Bradyrhizobium strains. Plant Science 159, 257-264.

Rosolem C.A., Foloni J.S.S., Tiritan C.S., 2002. Root growth and nutrient accumulation in cover crops as affected by soil compaction. Soil Till. Res., 65: 109-115.

Staples, K. D., Hamama, A. A., Knight-mason, R., & Bhardwaj, H. L. (2017). Alkaloids in White Lupin and Their Effects on Symbiotic N Fixation, 9(6), 13–21. https://doi.org/10.5539/jas.v9n6p13.

Stepkowski T., Moulin L., Krzyzanska A., McInnes A., Law I.J., Howieson J., 2005. European origin of Bradyrhizobium populations infecting lupins and serradella in soils of Western Australia and South Africa. Appl. Environ. Microbiol., 71: 7041-7052.