Plant Cell Biotechnology and Molecular Biology 21(57&58):78-85; 2020 ISSN: 0972-2025

IMPROVED COWPEA (*Vigna unguiculata* L. WALP.) RHIZOBIUM TOLERATE TO DROUGHT AND SALINITY CONDITIONS USING CO-INCULATION

**KADHIMIYAH JAWAD ABDULLAH Al-MANSOR*
AND ABDAL ZAHRA TAHATHAHER**
Department of Soil Sciences and Water Resources, College of Agriculture, Basra University, Iraq [KJAAM, AZT].
[*For Correspondence: E-mail: kadmajwad8@gmail.com, abdalzahrataha503@gmail.com]

Article Information

Editor(s):
(1) Dr. Afroz Alam, Banasthali University, India.
Reviewers:
(1) C. Appunu, ICAR-Sugarcane Breeding Institute, India.
(2) Nuryazmeen Farhan Haron, Universiti Teknologi Mara, Malaysia.

Received: 04 September2020
Accepted: 11 November 2020
Published: 30 November 2020

Original Research Article

ABSTRACT

The study was conducted to find out the effect of adding a mixture inoculation and its tolerance to salinity and lack of moisture on the growth of cowpea plant and its absorption of N , P and K nutrients, After planting and growing cowpea plant for a period of 60 days, then the plants were harvested and dried at 70°C for 48 hours, and their dry weight was taken, then milled, sifted and digested , then N, P and K were measured, and the roots of the plant was separated using a light water current and were count the number of root nodules for each plant.

The results showed that:

1- The mixed inoculation treatment (I_7) increased the rate of dry weight, root nodule number and nitrogen, phosphorus and potassium concentration in cowpea plant.

2- An increase in salinity levels reduced the rate of dry weight, average root nodule number, nitrogen, phosphorus and potassium concentration in cowpea plant and the same results were obtained with reducing moisture condition.

3- The mixed inoculation were increase the tolerance of cowpea plant to the salinity and drought condition led to an increase in N , P , K absorption and enhances the root nodules formation and increase dry weight .

Keywords: Rhizobia; cowpea; tolerance; salinity; drought.

INTRODUCTION

Some legumes are characterized by their ability to tolerate conditions of climatic extremism and salt stress, but their susceptibility to infection the *Rhizobium* and the formation of root nodules is very weak [1]. Scientists have estimated that there is evidence of nitrogen coexistence with legumes in the world about (155) million tonnes .year^{-1} [2,3]. An attempt has been made to increase the efficiency of the *Rhizobium*bacteria, which is the most important bacteria that scientific research seeks to use in the agricultural field in the range of Bio fertilizers with the highest possible efficiency, which is a biological method that does not result in any environmental pollution. Which is considered one of the methods of microbial technology in the field of biological fertilizers [4,5,6]. Friel et al. [1] found that it is not possible to completely rely on the native *Rhizobium* bacteria to infect roots of leguminous plants , As most of the soils contain a less number of local *Rhizobium* bacteria with high efficiency in causing infection, it is common that 1-25% of the local *Rhizobium* bacteria in the soil are characterized by a low ability to cause infection and 50% of them are characterized by medium capacity and 25% of them are distinguished. With high efficiency in causing infection [7] also, the slow-growing *Rhizobium* bacteria are less tolerant of the extremes of biotic and abiotic conditions than the rapidly growing *Rhizobium* bacteria [2]. Therefore, the link between Mycorrhizae and legumes is one of the beneficial relationships that can enhance plant resistance to biotic and abiotic stresses [2]. This relationship is through which the exchange of two different compounds is controlled: nitrogen (N) and carbon (C), as plant supplies microorganism with carbohydrates, which are used as a source of carbon and energy [8], and in return, the microorganisms stimulates the N2 fixation process by the root nodules of legumes [9] Also, fixation of atmospheric nitrogen by *Rhizobium* requires presence of phosphorus in optimum quantities in leguminous plants [10].

The using of bio-fertilizers such as Mycorrhizal fungi and phosphate-dissolving bacteria in leguminous crops is a promising technique that could be an alternative source of nitrogen and phosphorus to support the efficiency of rhizobium bacteria [11]. In addition, the co-inoculation system for cowpea plants was applied in the field using *Pseudomonas*, mycorrhizae, and *Rhizophagus* fungi, alone or in combination . Inoculation of seeds with (*P. libanensis* + *R. iriformis*) (PMR) led to significant increases in plant dry weight (76%), and the number of nodules , seed count per plant (52% and 56%, respectively) and grain yield (56%) in comparison with non-inoculated control treatments. The fat content also increased Grains increased by (25%) in PMR-inoculated plants compared to control, and high soil organic matter and low pH were observed in PMR treatment [12]. Method that relies on inoculating seeds, soil or both with one or more of beneficial microorganisms as co-inoculation behaves defending against the hostile microbes and pathogen and supplies it with the necessary nutrients [13].

In our current research, this microorganism was represented in a bacterium (*Pseudomonas fluorescence*) that produces the antibiotic, which is characterized by an anti-biotic relationship in its relationship with other soil microbes, and *Glomusmosseae,* which provides its haves and provides *Rhizobium* with nutrients and moisture, and can reduce the effect of pollution with heavy elements.

The research aim to use efficient isolate of *Rhizobium* in a companion inoculate that includes *Pseudomonas* and Mycorrhizae fungi for the purpose of enhancing the growth and efficiency of *Rhizobium* bacteria in infection of cowpea plant and promote its growth under different salt and moisture conditions.

MATERIALS AND METHODS

This experiment was conducted in a Green house, as plastic pots with a capacity of 5 kg soil were prepared. Surface sterilized each pot with 95% ethanol. Then inside each pot a piece of glass wool was placed on the bottom. Spread a quantity of sterile washed gravel on top of 1-0.5 cm diameters with a depth of 2 cm, then filled in each pot ofKteeban soil samples with electrical conductivity 3.99 dS. m^{-1} was salted with drainage water to salinity levels 6, 12, 24 dS . m^{-1}, and the original soil was washed with RO water up to the salt level 3 dS. m^{-1} .A total of 72 containers of 18 pots were filled with a level of salt from the levels prepared above at a rate of 5 kg. pot^{-1} .The mixed inoculation treatment I_7 (R_6+P+M) was added (as R_6 is an isolate of Rhizobium bacteria isolated from the roots of the cowpea plant, and P is an isolation of *Pseudomonas* bacteria and M aremycorrhizal fungi).

Pots were inoculated with Mycorrhizae by placing 100 g of inoculant under the surface of the potted soil, 5 cm deep, and another 100 g of inoculation was mixed with the surface layer of the soil and left the pots without inoculation as control (I_0). Homogeneous and superficially sterilized cowpea seeds were inoculated with rhizobia (isolate R_6) and pseudomonas P [14] at a density of 0.85 * 10^8cfu .ml^{-1} of the inoculum [15,16] by soaking them in the inoculum suspension for half an hour with the addition of gum Arabic to increase the adhesion of bacteria to the seeds, then the inoculated seeds were left to dry for an hour and a half away from direct sunlight [17]. Added with four prepared salt levels (S_1, S_2, S_3, S_4) and three moisture levels (1, 1/2 , 1/4) of soil field capacity of M_1, M_2, M_3. After sowing, the pots were weighed daily in order to compensate for the loss in weight for the duration of the experiment by adding water.

To each pot was added a triple superphosphate fertilizer of 100 kg . ha^{-1} P_2O_5 (47%), potassium sulfate fertilizer of 100 kg . ha^{-1} K_2O (52%) and urea fertilizer of 30 kg. ha^{-1} N (46%) which represented 75% of the fertilizer recommendation for nitrogen Additive as activated dose and organic fertilizer was added in the amount of 5 tons. ha^{-1}.

The experiment lasted for 60 days, after which the plants were harvested and dried at 70°C. The dry weight was estimated and milled, then digested and its content of N, P and K were estimated [18]. Then the root groups of the plants were carefully removed so that all parts of the root system could be seen. A weak stream of tap water was directed to the soil. Then the entire root system of each plant in each pot was examined and the number of root nodules were recorded.

RESULTS AND DISCUSSION

It is noticed from Table 1 that the inoculation, level of humidity and the level of salinity have a significant effect on increasing the dry weight of cowpea plant , as all inoculation and moisture level interaction treatments exceeded the comparison treatment and the recorded values were(15.91, 13.49, 9.99) g.$pot^{-1}$for I_7M_1 , I_7M_2 and I_7M_3 respectively, while the comparison treatment was (8.08, 8.33, 6.33) g .$pot^{-1}$with I_0M_1 , I_0M_2 and I_0M_3, respectively .This difference in the dry weight between the comparison treatment and the inoculated treatment may be due to the fact that these organisms have the ability to increase supply of nutrients to the plant, which leads to the elongation of the plant and its increase in weight [19]. The increase in level of salinity led to a decrease in average dry weight of shoot, reaching 15.99, 11.49, 7.99 and 5.94 g for each of S1, S2, S3 and S4 respectively, that increase of salts leads to a negative effect on the plant by its direct and secondary effect. The high levels of salinity lead to lower levels of plant nutrient supplies due to the absorption process that occurs in the soil solution , thus reducing root growth and a direct effect on plant growth, height and weight [20]. The interaction between the inoculation treatments and the salinity and moisture levels showed a significant effect on the dry weight of the shoot, as treatment ($I_7+S_1+M_1$) gave the highest rate of 29.66 g, followed by treatment ($I_7S_1M_2$) reached 26.33 g.pot^{-1}, while the comparison treatment ($I_0+S_4+M_3$) reached to 4.00 g.pot^{-1}.

Table 1. The effect of adding biological Inoculation with different salt and moisture levels on the average dry weight of shoot g.pot^{-1}

AverageI*M	Salinity level				Moisture level	
	S_4	S_3	S_2	S_1		Inoculants
8.08	8.00	7.00	9.00	8.33	M1	I_0
8.33	7.00	6.66	9.33	10.33	M2	
6.33	4.00	8.00	5.66	6.66	M3	
15.91	5.66	13.00	15.33	29.66	M1	I_7
13.49	6.00	8.00	13.66	26.33	M2	
9.99	5.00	5.33	16.00	14.66	M3	
	5.94	8.00	11.50	16.00	Average S	
	ISM= 4.767 , IM= 2.383 , S=1.946				LSD	

The treatment (I_7+S_2+M_3) and (I_7+S_1+M_3) were 16, 14.66 g.pot^{-1} due to inoculation with cowpea *Rhizobium* that secrete lipopolysac-charides, the most important compound for osmatic resistance and helping the bacteria to fixing nitrogen, this was agreement with [2] and [21] they found that some strain of *Rhizobium* were enter inside the root tissues as low molecular weight organic compounds, this mechanism help the organisms adapt to high salinity.

It is noted from Table 2 that the interaction between inoculated with I_7 treatment and levels of moisture were affected significantly on nodule formation as all inoculated treatments exceeded the comparison treatment and the values were (18.833, 8.499 and 4.416) nodules.pot^{-1} for each of I_7M_1, $I_7M_2$and I_7M_3 respectively, while in the comparative treatment 1.999, 0.749 and 0.000 nodules.pot^{-1} for I_0M_1, $I_0M_2$and I_0M_3 respectively. This difference in the total number of root nodules between the comparison and the inoculated treatment may be due to the fact that these organisms have the ability to supply the nutrients of the plant significantly, which leads to the formation of root nodules, as well as the joint inoculation increases the length and mass of the root, and thus the number of larger sites of infection and the formation of nodules by *Rhizobium* bacteria [22].

The increase in the salinity level has led to a decrease in the average number of total root nodules, reaching 15.55, 4.944, 1.944, and 0.555 nodules.pot^{-1} for S_1, S_2, S_3 and S_4 respectively. As salinity, extreme temperatures and insufficient or excessive soil moisture impose restrictions in the ability to supply nitrogen, phosphorus and potassium to the host plant by the *Rhizobium* bacteria. Salinity which rise a water stress that affects the rate of photosynthesis or may be affect the formation of root nodules directly [2]. The triple interaction between the inoculation and levels of salinity and humidity showed a significant effect on the characteristic of the total number of root nodules. As the treatment I_7 with the level of salinity of S_1 and the level of moisture of M_1 gave the highest rate of 54,333 nodules.pot^{-1} , while the comparison treatment with the level of salinity of S_4 and the level of moisture of M_3 the lowest value 0.000 nodules.pot^{-1}.

The reason for the decrease in the total root nodule rate with the low moisture content of the soil is due to the decrease in the number of thread infection formed on the root capillaries, and thus less root nodules formation [2]. These results are consistent with the previous findings [23].

The result in Table 3 showed that the interaction between inoculated with mixed isolation (I_7) and levels of moisture were significant effect on nitrogen present in cowpea plant. The inoculated treatments exceeded the non-inoculation treatment with 2.83, 2.56and 2.13% for each of I_7M_1, I_7M_2 and I_7M_3 respectively. The non-inoculated treatment recorded 1.72, 1.56 and 1.47% in treatmentsI_0M_1, $I_0M_2$and I_0M_3respectively . This difference in the nitrogen concentration in the plant between the comparison and inoculated treatment may be due to the fact that these organisms have the ability to significantly supply the plant nutrients [5]. Bacterial inoculation lead to an increase in the supply of nutrients involved in the process of plant building, plant elongation and increase in growth [1]. The increase in the salinity level led to a decrease in the average nitrogen concentration in the plant, reaching 2.72, 2.25, 1.77, and 1.44 % for each of S_1, S_2, S_3 and S_4 respectively. The increase in salt levels leads to a negative effect on the plant. The high levels of salinity lead to lower levels of nutrient availability for the plant due to the osmotic stress and non- equilibrium process that occurs in the soil solution , thus reducing the growth of roots and the direct effect on the growth, height and weight of the plant .The interaction between the mixed inoculation and the levels of salinity and moisture showed a significant effect on nitrogen concentration in the plant, as the treatment (I_7+ S_1+ M_1) gave the highest rate of 4.03%, while the comparison treatment (I_0 + S_4 + M_2) reached the lowest value 1.28%. The result showed not significant differences between treatments (I_7+S_2+M_2) and (I_7+S_1+M_2) and between (I_7+S_2+M_3) and (I_7+S_1+M_3). It indicated that the inoculation with mixed inoculation increased the plant tolerances to the salinity and drought conditions of soil.

Table 2. The effect of adding biological Inoculation with different salt and moisture levels on the average number of root nodules (nodules.pot^{-1})

AverageI*M	Salinity level				Moisture level / Inoculants	
	S_4	S_3	S_2	S_1		
1.999	.000	.333	3.666	4.000	M1	I_0
0.749	.000	.000	1.666	1.333	M2	
0.000	.000	.000	.000	.000	M3	
18.833	.333	7.666	13.000	54.333	M1	I_7
8.499	1.666	2.000	8.666	21.666	M2	
4.416	1.333	1.666	2.666	12.000	M3	
	0.56	1.94	4.94	15.56	AverageS	
	ISM= 4.537 , IM= 2.269 , S=1.852				LSD	

Table 3. The effect of adding biological Inoculation with different salt and moisture levels on the rate of nitrogen in plant (%)

AverageI*M	Salinity level				Moisture level / Inoculants	
	S_4	S_3	S_2	S_1		
1.72	1.40	1.50	1.76	2.23	M1	I_0
1.56	1.28	1.46	1.60	1.93	M2	
1.47	1.36	1.30	1.43	1.80	M3	
2.83	1.50	2.56	3.26	4.03	M1	I_7
2.56	1.60	2.10	3.06	3.50	M2	
2.13	1.53	1.70	2.43	2.86	M3	
	1.441	1.773	2.256	2.725	Average S	
	ISM= 0.4432 , IM= 0.2216 , S=0.1810				LSD	

The result in Table 4 showed the double interaction between inoculation and moisture levels had effect significantly on phosphorous concentration as all the inoculated treatments exceeded the non-inoculation treatment with 0.30, 0.27and0.21 % for each of I_7M_1, I_7M_2 and I_7M_3 respectively, while the non-inoculation treatmentI_0M_1, I_0M_2, I_0M_3recorded 0.19, 0.19 and 0.14% and that effect of treatment increased by 57.9, 42.1 and 50 % compared with out inoculation respectively.

The difference in phosphorus concentration in the plant between the inoculation and non-inoculation treatment were due to the ability of these organisms to supply nutrients to the plant and improved the moisture content [24].

Increase in level of salinity has led to a decrease in average concentration of phosphorus in plant, reaching 0.31, 0.24, 0.19, and 0.14% for S_1, S_2, S_3 and S_4 respectively, as salinity, impose restriction on ability to supply phosphorus to host plant by the *Rhizobium* bacteria [2].

The triple interaction between three factors showed that the treatment ($I_7+S_1+M_1$) and ($I_7+S_1+M_2$) gave the highest rate of 0.41% , while the comparison treatment ($I_0+S_4 + M_3$)reached the lowest value 0.09% which indicated that the combined inoculation is very beneficial even with an increased percentage of available phosphorous to plant [25].

It is noted from Table (5) , that the double interaction between inoculation treatment and moisture level were significant effect on potassium concentration in the plant, as all inoculated treatments exceeded the comparison treatment (2.04, 1.77 and 1.21)% for each of I_7M_1 , $I_7M_2$and I_7M_3 respectively , while it was0.51, 0.48and 0.43% in comparison treatment I_0M_1, I_0M_2 and I_0M_3 respectively.

This difference in potassium concentration in plants between the inoculation and non-inoculation treatment may be due to the fact that these organisms increase the absorption of nutrients, increase the rate of photosynthesis and tolerate abiotic stress [26].

The increase in the salinity level led to a decrease in the average potassium concentration in the plant, reaching 1.57, 1.42, 0.84, and 0.46% for each of S_1, S_2, S_3 and S_4, respectively.

Table 4. The effect of adding biological Inoculation with different salt and moisture levels on the rate of phosphorus in plant (%)

AverageI*M	Salinity level				Moisture level / Inoculants	
	S_4	S_3	S_2	S_1		
0.19	0.11	0.19	0.21	0.27	M1	I_0
0.19	0.11	0.15	0.23	0.27	M2	
0.14	0.09	0.12	0.15	0.23	M3	
0.30	0.20	0.24	0.36	0.41	M1	I_7
0.27	0.19	0.23	0.27	0.41	M2	
0.21	0.16	0.22	0.24	0.25	M3	
	0.1458	0.1944	0.2472	0.3094	S Average	
	ISM= 0.04664 , IM= 0.02332 , S =0.01904				LSD	

Table 5. The effect of adding biological Inoculation with different salt and moisture levels on the rate of potassium in plant(%)

AverageI*M	Salinity level				Moisture level / Inoculants	
	S_4	S_3	S_2	S_1		
0.51	0.49	0.50	0.53	0.52	M1	I_0
0.48	0.45	0.49	0.50	0.49	M2	
0.43	0.43	0.45	0.43	0.42	M3	
2.04	0.51	1.76	2.93	2.96	M1	I_7
1.77	0.46	1.30	2.40	2.93	M2	
1.21	0.43	0.58	1.73	2.13	M3	
	0.461	0.846	1.42	1.575	S Average	
	ISM= 0.2002 , IM= 0.1001 , S=0.0817				LSD	

The triple interaction between three factors showed a significant effect on potassium concentration in the plant, as the treatment (I_7 + S_1 + M_1) gave the highest rate of 2.96%, followed by the treatment (I_7 +S_1+ M_2) and (I_7 + S_2 + M_1) which reached to 2.93%, while the treatment (I_0+S_1+M_3) reached the lowest value 0.42% . The reason for the significant increase in the concentration of potassium in the cowpea plant, even with the increase in salinity and the reduction of the moisture content, is mainly due to the role of the co-inoculation, as the presence of mycorrhizal fungi reduces the concentration of salts by providing appropriate moisture [25]. And that the pseudomonas bacteria help with phosphorous and some antibiotics that protect the plant from pathogens and thus encourage the process of root nodule formation and absorption of the nutrients necessary to complete the biological processes inside the plant [2].

CONCLUSION

This study conclude that mixed inoculation led to improve tolerance of cowpea *Rhizobium* to salinity and drought condition and enhances the root nodule formation, and plant growth and its N, P, K absorption.

ACKNOWLEDGMENTS

The authors thank the staff of the Department of Soil science and Water Resources, College of Agriculture , University of Basrah for supporting this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Friel CA, Friesen ML. Legumes modulate allocation to rhizobial nitrogen fixation in response to factorial light and nitrogen manipulation. Front. Plant Sci. 2019;10: 1316.
DOI: 10.3389/fpls.2019.01316
2. Zahran, HH Rhizobium–legume symbiosis and fixation under severe conditions and in an arid climat. Department of Botany, Faculty of

Science, Beni-suef, 62511 Egypt. Microbiology and molecular Biology reviews, 1999,1092-2172/99 /$04.0010. American Society for Microbiology. 1999; 63(4):968-989.

3. Al-Tawaha ARM, Al-Tawaha A, Sirajuddin SN, McNeil D, Othman YA, Al-Rawashdeh IM, Amanullah, Imran, Qaisi AM, Jahan N, Shah MA, Khalid S, Sami R, Rauf A, Thangadurai D , Sangeetha J, S Fahad, Youssef RA, Al-Taisan WA, Al-Taey DKA. Ecology and adaptation of legumes crops. IOP Conf. Series: Earth and Environmental Science. 2020;492:012085.
4. Al-Sherif EM. Ecological studies on the flora of some aquatic systems in Beni-suef district. M.Sc. Thesis. Cairo university (Beni-Suef Branch), Beni-Suef, Egypt; 1998.
5. Thilakarathna MS, Chapagain T, Ghimire B, Pudasaini R, Tamang BB, Gurung K, Choi K, Rai L, Magar S, Bishnu BK, Gaire S, Raizada MN. evaluating the effectiveness of rhizobium inoculants and micronutrients as technologies for nepalese common bean smallholder farmers in the real-world context of highly variable hillside environments and indigenous farming practices . Agriculture J. 2019;9: 20.
6. Al-Taey DKA, Majid ZZ. The activity of antioxidant enzymes and NPK contents as affected by water quality, Kinetin, Bio and organic fertilization lettuce (*Lactuca sativa* L). The Iraqi Journal of Agricultural Science. 2018;49(3):506-518.
7. Rahman M, Islam M, Bhuiyan M, Khanam D, Hossain A, Rahman A. Effect of rhizobia inculum with and with out chemical fertilizer on chikpen in haqeps. Bangladesh . J. Agric. Sci. 1994;21(2): 273-277.
8. Slomy AK, Jasman AK, Kadhim FJ, AL-Taey DKA, Sahib MR. Study Impact of Some Biofactorson The Eggplant *Solanum melongena* L. vegetative characteristics under glass houses conditions. Int. J. Agricult. Stat. Sci. 2019; 15(1):371-374.
9. Rydlova J, Puschel D. Arbuscular mycorrhiza, but not hydrogel, alleviates drought stress of ornamental plants in peat-based substrate. Pplied Soil Ecology. 2020; 146:1033942.
   Available:https://doi.org/10.1016/j.apsoil.2019.103394
10. White J, Prell J, James EK, Poole P. Nutrient sharing betweensymbionts. Plant Physiol. 2007;144:604–614.
11. Parween T, Bhandari P, Jan S, uzzafar M, Fatma T, Raza SK. Role of bioinoculants as plant growth-promoting microbes for sustainable agriculture. Agriculture; 2017. DOI: 10.1007/978-981-10-5589-8_9
12. Pereira S , Mucha Â, Gonçalves B, Bacelar E, Látr A, H Ferreira, Oliveira I, Rosa E, Marques G. Improvement of some growth and yield parameters of faba bean (*Vicia faba*) by inoculation with rhizobium laguerreae and arbuscularmycorrhizal fungi. Plant Sciences, Sustainable Farming Systems and Food Quality. 2019;70(7).
13. Rocha I, Souza-Alonso P, Pereira G, Ma Y, Vosátka M, Freitas H, Oliveira RS. Using microbial seed coating for improving cowpea productivity under a low-input agricultural system. J. The Scinceof FoodandAgricaltur. 2019;100(3).
14. Shalini, Srivastara R. Screening for antifungal activity of pseudomonas fluorescens against phyto pathogenic fungi. Joural of Microbiology. 2008;5(2).
15. Beck DP, Materon LA, F Afandi. Practical rhizobium legume technology manual technical. ICARDA, Syria. 1993;19.
16. Date, RA. Microbiological problems in the inoculation and nodulation of legumes. Plant and Soil. 1970;32:703-725.
17. Date RA. Inoculated legumes in cropping systems of the tropics. Field crops Res. 2000;65:123–136.
18. FNCA forum for nuclear cooperation in asia. Bio fertilizer manual. Japan Atomic Industrial Forum (JAIF), Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan. FNCA; 2006.
   Available:http://www.fnca.jp/english/index.html
19. Minaxi J Saxena S Chandra, Nain L Synergistic effect of phosphate solubilizing rhizobacteria and arbuscularmycorrhiza on

growth and yield of wheat plants. Journal of Soil Science and Plant Nutrition. 2013; 13(2):511-525.
20. Santachiara G, Salvagiottib F, Rotundoa JL. Nutritional and environmental effects on biological nitrogen fixation in soybean: A meta-analysis. Field Crops Research. 2019; 240;106–115.
21. Vriezen JAC, de Bruijn FJ, Nusslein K. Responses of rhizobia to desiccation in relation to osmotic stress, oxygen, and temperature. Applied and Environmental Microbiology. 2007;73:3451–59.
22. Korir H, Mungai NW, Thuita M, Hamba Y Masso C. Co-inoculation effect of rhizobia and plant growth promoting rhizobacteria on common bean growth in a low phosphorus soil. Front. Plant Sci. 2017;8: 141.
DOI: 10.3389/fpls.2017.00141
23. Figueiredo B, Burity HA, de França FP. Water deficit stress effects on N2 fixation in cowpea inoculated with different *Bradyrhizobium* strains. Can. J. Plant Sci.1998
Available:www.nrcresearchpress.com by 185.180.63.2 on 12/29/19
24. Kasper S, Christoffersen B, Soti P, Racelis A. Abiotic and biotic limitations to nodulation by leguminous cover crops in south texas. Agriculture J. 2019;9:209.
25. Hack CM, Porta M, Schäufele R, Grimoldi AA. Arbuscular mycorrhiza mediated effects on growth, mineral nutrition and biological nitrogen fixation of Melilotusalba Med. in a subtropical grassland soil. Applied Soil Ecology. 2019; 134;38-44.
DOI: org/10.1002/jsfa.10117
26. Lopes EAP, Silva A, Mergulhao A, Silva E, Santiago A, Figueiredo M. Co-Inoculation of growth promoting bacteria and glomusclarum in micropropagated cassava plants. Rev. Caatinga Mossoró. 2019;32(1): 152–166.