

Original Research Article

Evaluation of two chickpea (*Cicer arietinum* L.) cultivars in response to three *Rhizobium* strains at River Nile State, Sudan

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Abstract

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The performance of two chickpea in response to three *Rhizobium* strains was evaluated. The main objective of the work was to study the effect of inoculation with different *Rhizobium* strains on yield, nodulation and other agronomic characteristics of two chickpea cultivars and examine the interaction. In this two factor factorial field experiment of three *Rhizobia* strains (ENRRI 8, USDA3100 and TAL 620) along with uninoculated (control) were compared on two chickpea cultivars (Burgeig and Salwa) and the eight treatment combinations were arranged in randomized complete block design with three replications. The inoculation increased the plant traits of the two cultivars. Inoculated plants gave higher nodule dry weight mg plant⁻¹, seed yield g plant⁻¹, number of filled pods plant⁻¹, seed number plant⁻¹ compared to un-inoculated plants. The various *Rhizobium* strains differed in their effects. USAD3100 strain was found to be the most efficient in increase yield and yield components. TAL 620 strain was most effect in delaying days to 50 flowering and days to 90% maturity and gave tall plant height. The two cultivars responded differently to inoculation. Salwa cultivar responds to TAL 620 strain, whereas Burgeig cultivar responds to USDA3100 strain. Salwa cultivar was more efficient in *Rhizobium* production. On the basis of analysis of correlation coefficients, it was found that number of filled pods plant⁻¹, seed number plant⁻¹ were the best criterion for selection of most effective legume-*Rhizobium* associations.

Keywords: Chickpea cultivars, Correlation, Nodulation, Plant traits, *Rhizobium* strains, Sudan.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important leguminous crop with high nutritive value and source of protein. It is considered to sustain cropping system productivity due to its ability to fix atmospheric nitrogen. It has highly specific symbiotic association, with a unique group of rhizobia necessary for formation of nodules and nitrogen fixation (*Rhizobium leguminosarum* subsp. *Ciceri*). Absence of suitable strains, small population size and poor survival of rhizobia cause problems in nodules formation (Kantar *et al.*, 2007).

Rhizobium symbiosis with legumes species is of special importance, producing 50% of 175 million ones of total biological N fixation annually worldwide (Sarıoğlu

et al., 1993). Chickpea and *Rhizobium leguminosarum* subsp. *Ciceri* association annually produce up to 176 kg N ha⁻¹ depending on cultivar, bacterial strain, and environmental factors (Rupela and Kumar Rao, 1987; Beck *et al.*, 1991). Increasing and extending the role of biofertilizers such as *Rhizobium* would reduce the need for chemical fertilizers and decrease adverse environmental effects. Therefore, in the development and implementation of sustainable agriculture techniques, biofertilization is of great importance in alleviating environmental pollution and the deterioration of nature (Elkoca *et al.*, 2008).

Symbiotic nitrogen fixation (SNF) resulting from

mutual beneficial interaction between chickpea and soil nodule bacteria (rhizobia) provides increase to N fertilization and additionally, does not cause any hazard to the environment. Thus chickpea depends on its symbionts for a large part of its nitrogen requirements for effective growth and dry matter production. Therefore, the success of this crop in the country lies on its efficient symbiosis with N₂-fixing bacteria. The variable extent of nitrogen fixation by chickpea cultivars is probably due to differences in symbiotic effectiveness of rhizobial strains and their compatibility. Selections of host cultivar-compatible inoculants have been recognized as an important method for increasing nitrogen fixation in chickpea. Nitrogen fixing potential of chickpea genotypes can be increased significantly by rhizobial inoculation. Grain yield of chickpea increased considerably with rhizobial application (Khattak *et al.*, 2006). Romdhane *et al.* (2008) also reported that chickpea yield can be enhanced by inoculation with competitive rhizobia and is especially economical promising to increase chickpea production. Artificial seed inoculation of chickpea in those soils lacking native effective rhizobia is a very useful practice for improving root nodulation and yield of the crop (Khattak *et al.*, 2006, Muhammad *et al.*, 2010).

Using high yielding varieties of chickpea along with use of effective rhizobial strains can enhance the yield. There is a good possibility to increase its production by exploiting better colonization of the roots and rhizospheres through application of effective nitrogen fixing bacteria to the seed or to the soil. This can minimize uses of nitrogenous fertilizer which is very costly and the extensive use of chemical fertilizers in agriculture is currently under debate due to environmental concern and fear for consumer' health. Consequently, there has been a growing level of interest in environmentally friendly sustainable agricultural practices and organic farming systems (Rigby and Caceres, 2001; Lee and Song, 2007). Therefore, the present study was undertaken to study the response of different chickpea cultivars to Rhizobium strains, which are superior in nodulation, plant development and increase seed yield.

MATERIALS AND METHODS

The experiment was conducted for two seasons (2008-2010) at Hudeiba Research Station, Northern Sudan, to study the effect of Rhizobium (*Rhizobium ciceri*), Cultivar and their interaction on yield and yield components of chickpea (*Cicer arietinum*). Two cultivars; Salwa (FLIP 89-82c) and Burgeig (ICCV 91302) and four treatments include un-inoculated control and three inoculated Rhizobium strains of bacteria symbiosis (ENRRI8, USDA3100 and TAL 620) were used, strains were provided by the Environment and Natural Resources Research Institute (ENRRI), the National Centre for

Research, Khartoum, Sudan, strain ENRRI 8 was locally isolated, strain TAL 620 was obtained from NifTAL project, University of Hawaii, USA and strain USDA 3100 from USA Department of Agriculture. The experiment was laid out in factorial field experiment with two factors (four treatments and two cultivars). The eight treatment combinations were arranged in randomized complete block design with three replications.

Seeds were inoculated before planting by adding 40% (w/v) gum Arabic solution and mixed to wet all seeds then a charcoal-based inoculum of the required *Rhizobium* was added and mixed until all seeds were coated. Sowing date was at 16 November for the two seasons. Plot size was 5 × 3 m². The irrigation was every ten days. Planting space were 60 cm between ridges and 10 cm between plant holes with two seeds per hole. The insecticide Tracer was used for American boll worm management. Weed control was applied three times by used hand weeding.

Five randomly selected plants were uprooted at 50% flowering stage from each plot to record nodule wet weight and nodule dry weight.mg plant⁻¹ At harvest data were collected on seed yield g plant⁻¹, numbers of filled pods plant⁻¹, seeds number plant⁻¹ as average of five randomly selected plants. Data on days to 50% flowering, days to 90% maturity, plant height, and 100-seed weight was also taken. The collected data was analyzed by using statistical software, GenStat 12th edition (Package, 2009).

RESULT

Effects of cultivars, rhizobium inoculums and their interaction form analysis of variance for each season and the average on nodule dry and wet weights mg plant⁻¹, seed yield g plant⁻¹, yield components and vegetative traits is presented in Table 1. All strains were shown to induce nodules on the tested cultivars (Table 2). However, significant effect of inoculation treatments in the first season was found for number of filled pods plant⁻¹, whereas in the second season significant differences were obtained for nodule wet and dry weights mg plant⁻¹. Days to 50% flowering showed significant difference in two seasons. In overall basis, significant differences due to inoculation treatments were obtained for nodule wet and dry weights mg plant⁻¹, days to 50% flowering and days to 90% maturity, and number of seeds plant⁻¹ (Table 1). Remarkable increases for studied traits due to strains induction were shown in tables 2, 3 and 4. Chickpea cultivars differed significantly in the days to 50% flowering and number of filled pods plant⁻¹ in the first season, while in the second season significant difference was found for seed yield g plant⁻¹, nodule wet weight mg plant⁻¹. 100-seed weight showed significant difference for both seasons and for the average. On the average basis, significant differences due to cultivars were obtained for days to 50% flowering and number of filled pods plant⁻¹

Table 3. Continue

Burgeig	34	25	43	37	30	46	24.8	30.5	24.9
Salwa	51	46	36	55	50	40	25.1	33.2	31.8
LSD5%	5.9	11.4	6.1	11.1	10.3	7.2	2.5	1.1	1.3
CV%	23	26.5	26.4	37.9	22.4	28.4	10.2	4.4	7.7

Table 4. Means of vegetative traits of two chickpea cultivars evaluated in four Rhizobial inoculant treatments at Hudeiba for two consecutive seasons; 2008/09, 2009/10 and the average.

Treatment	Days to 50 % flowering			Days to 90 % maturity			Plant height (cm)		
	2008-09	2009-10	Mean	2008-09	2009-10	Mean	2008-09	2009-10	Mean
Control	47	44	46	110	101	105	34.5	55.1	44.8
ENRRI 8	45	47	46	110	102	106	35.2	54.1	44.7
TAL 620	54	50	52	112	106	109	36.4	57.1	46.8
USDA 3100	49	50	49	112	104	108	36.4	53.6	45.0
LSD5%	3	3	1.8	3.2	3.6	2.3	3.1	4.7	2.7
Cultivar									
Burgeig	43	55	45	112	110	107	36.2	35.1	44.9
Salwa	48	47	51	102	104	107	53.6	56.3	45.7
LSD5%	2	1.8	1.3	2.2	2.6	1.6	2.2	3.3	1.9
CV%	4.7	4.4	4.6	2.3	2.8	2.6	7.1	6.9	7.1

(Table 1). Significant difference due to cultivar x treatment interactions was found for seed yield g plant⁻¹, number of filled pods plant⁻¹ and days to 50% flowering in the first season and for days to 50% flowering in overall basis (Table 1).

Comparison of rhizobial strains revealed that the strain USDA3100 was most effective in nodule production gave significantly the highest nodule dry weight mg plant⁻¹ in the second season. For the average of two seasons, ENRRI 8 strain gave the highest nodule dry weight mg plant⁻¹, however TAL 620 strain gave the highest nodule wet weight mg plant⁻¹ (Table 2). All inoculated treatments enhanced chickpea seed yield g plant⁻¹ over uninoculated. Remarkable high seed yield g plant⁻¹ for the two seasons was observed with strain USDA3100 followed by TAL 620 and ENRRI 8 strains (Table 2). Generally inoculated treatments delay in days to 50% flowering and

days to 90% maturity. Strain TAL 620 gave the latest days to 50% flowering, days to 90% maturity, tall plant height and highest 100-seed weight. USDA3100 strain obtained the high number of seed and filled pods plant⁻¹ (Tables 3 and 4).

Chickpea cultivars differed in nodulation induced. Significantly the highest nodule wet weight mg plant⁻¹ and seed yield g plant⁻¹ was noted with Salwa cultivar in the second season. High nodule dry weight plant⁻¹ was also obtained in Salwa cultivar (Table 2). For yield component and vegetative traits significantly the highest 100-seed weight and tallest plant height was noted with cultivar Salwa in the second season (Tables 3 and 4). Nevertheless, high numbers of filled pods plant⁻¹ and seeds plant⁻¹ were obtained in Burgeig cultivar (Table 3).

Significant interaction in the first season showed

that Salwa cultivar gave the highest seed yield plant⁻¹ when inoculated by TAL 620 strain whereas, Burgeig cultivar gave the highest seed yield plant⁻¹ when inoculated by USDA 3100 strain. In overall basis, maximum seed yield was recorded for both cultivars in association with inoculants strain USDA 3100 (Figure 1). There was different response of cultivars for number of filled pods plant⁻¹ in first seasons. Burgeig cultivar revealed the highest filled pods plant⁻¹ response to USDA 3100 strain, whereas Salwa cultivar showed the highest filled pods plant⁻¹ response to TAL 620 strain (Figure 2). In first seasons, TAL 620 strain delay days to 50% flowering of Burgeig cultivar, while USDA 3100 strain delays days to 50% flowering for Salwa cultivar (Figure 3). Correlation coefficients among different traits for chickpea- Rhizobium treatments are presented in (Table 5). Most of traits showed non-significant

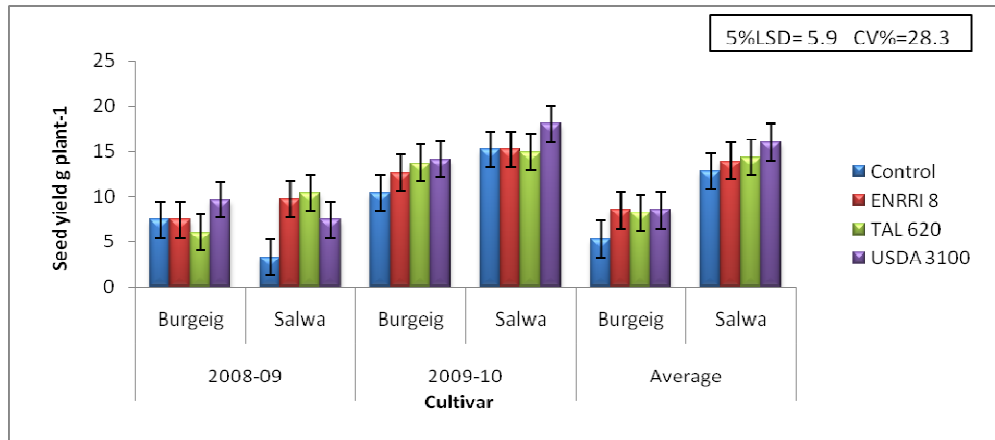


Figure 1. Interaction effects of cultivars and rhizobial inoculant on seed yield gram per plant of chickpea for two seasons and the average.

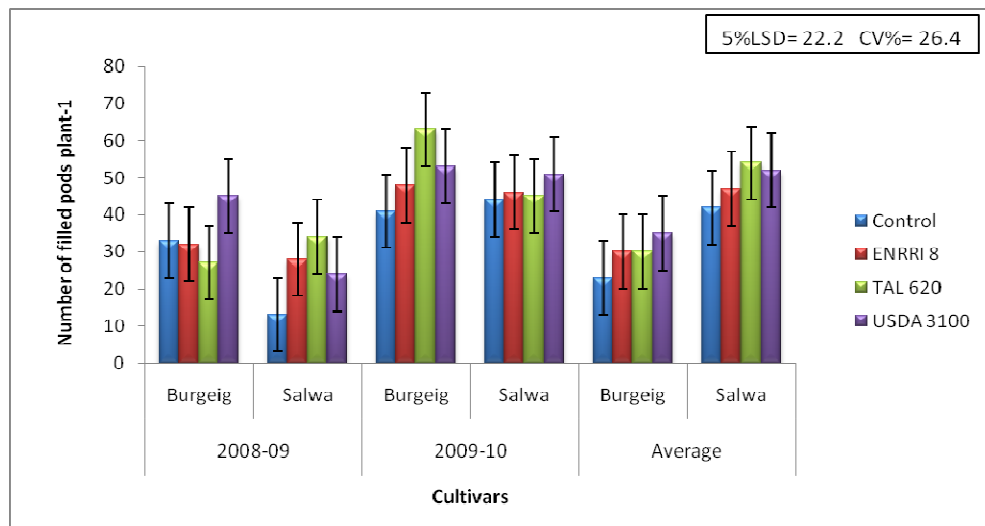


Figure 2. Interaction effects of cultivars and rhizobial inoculant on number of filled pods of chickpea for two seasons and the average.

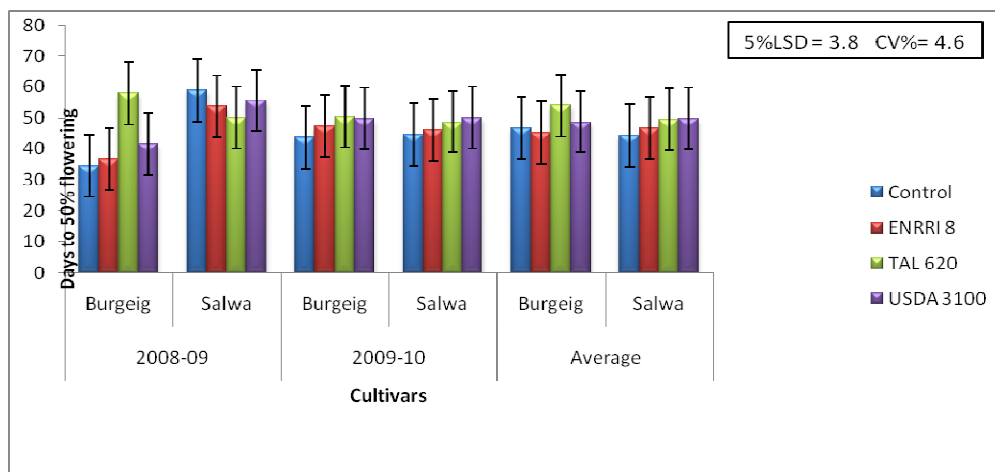


Figure 3. Interaction effects of cultivars and rhizobial inoculant on DAYS to 50% flowering of chickpea for two seasons and the average.

Table 5. Correlation coefficients between traits in two chickpea cultivar evaluated in four *Rhizobium* treatments in two seasons 2008/09 (S1) and 2009/10 (S2) at Hudeiba.

Traits		Nodules dry weight plant ⁻¹	Nodules wet weight plant ⁻¹	Seed yield g plant ⁻¹	Number of filled pods plant ⁻¹	Number of seeds plant ⁻¹	100-seed weight (g)	Days to 50 % flowering	Days to 90 % maturity
Nodules wet weight plant ⁻¹	S1	0.948**							
	S2	0.848**							
Seed yield g plant ⁻¹	S1	0.969**	0.894**						
	S2	0.368	0.498**						
Number of filled pods plant ⁻¹	S1	0.826**	0.722**	0.853**					
	S2	0.357	0.599**	0.648**					
Number of seeds plant ⁻¹	S1	0.667**	0.583**	0.703**	0.803**				
	S2	0.325	0.510**	0.760**	0.875**				
100-seed weight (g)	S1	-0.123	0.003	-0.047	-0.389	-0.232			
	S2	0.211	0.235	0.324	-0.217	-0.289			
Days to 50 % flowering	S1	-0.304	-0.142	-0.236	-0.493**	-0.232	0.651**		
	S2	0.36	0.508**	0.320	0.479**	0.600**	-0.161		
Days to 90 % maturity	S1	-0.015	-0.054	0.064	0.241	0.306	-0.234	-0.065	
	S2	0.27	0.647**	0.384	0.446*	0.484**	0.131	0.573**	
Plant height (cm)	S1	0.294	0.237	0.262	0.272	0.41	-0.248	-0.094	0.573
	S2	0.101	0.469*	0.283	0.364	0.275	0.202	0.098	0.735**

relationship. Seed yield correlated positively and significantly with nodules dry and wet weight per plant⁻¹. The three traits were positively and significantly correlated with number of filled pods plant⁻¹ and seeds number plant⁻¹.

DISCUSSION

In the present study cultivars showed nodulation in uninoculated treatment that confirming the presence of existing indigenous rhizobia in the soil. The seed inoculation with rhizobium significantly increased the nodules and seed yield g plant⁻¹. After inoculation, seed yield g plant⁻¹ increased at the rate of 19% and 20 % and 26 % for ENRRI 8 strain, TAL 620 strain and USDA 3100 strain respectively, over the uninoculated chickpea cultivars. The nodules dry weight plant⁻¹ was increased at rate of 35% and 28 % and 33 % for ENRRI 8, TAL 620 and USDA 3100

respectively, over the uninoculated chickpea cultivars. However, USDA 3100 strain was more efficient to increase seed yield g plant⁻¹. Bhuiyan et al. 2008 found that inoculated plants gave significantly higher nodule number, nodule weight, stover yield and seed yield compared to uninoculated control. Khanam et al. (1994) reported that inoculation with *Rhizobium* strains gave higher nodule number, nodule dry weight, stover yield and seed yield compared to uninoculated plants. Bhuiyan et al. (1998) found that *Rhizobium* inoculation increased nodulation and seed yields upto 35%. Gupta and Namdeo (1996b) found that seed inoculation with *Rhizobium* increased chickpea seed yields by 9.6-27.9%.

Effectiveness of strains on percent improvement in seed yield was low. Appunu et al. 2008 found that the efficiency of *Rhizobium*-legume symbiosis when grown under field conditions was adversely affected due to various

climatic and edaphic factors. The possible reason for significant increase in the nodules plant⁻¹ might be the increased rhizobial population per gram of soil. Due to increased rhizobia in the soil, the nodules plant⁻¹ increased which may possibly result in greater nitrogen fixation and eventually the yield components might be influenced positively which resulted in significant increase in the seed yield g plant⁻¹. A similar promotive effect of inoculation on nodules and seed yield g plant⁻¹ in chickpea was also observed by Akhteret al. (2013); Bhuiyan et al. (2008); Romdhane et al. (2008); Tellawiet al. (2007); Khatkhet al. (2006). There was increased in number of filled pods plant⁻¹ and number of seeds plant⁻¹. Better effect of rhizobium inoculation on seed yield and yield components of common Vetch (*Vicia sativa* L.) were also shown by Albayrak et al. (2006).

Significant differences were recorded in the nodule wet weight mg plant⁻¹, seed yield g plant⁻¹, number of filled pods plant⁻¹ and days to 50%

flowering among the cultivars. This confirmed that these cultivars showed different response to rhizobia. Khanam *et al.* (1994) in Bangladesh and Gupta and Namdeo (1996a) were also reported varied in nodulation and yield of chickpea due to use of different varieties. The reason of this difference is the interaction between bacteria (*Rhizobium ceceri*) and genotype of plant. The variation in nodulation among chickpea genotypes have also been reported by Tellawiet *al.* (2007), Mensah and Olukoya, (2007) and Gallaniet *al.* (2005). Similarly, Gul *et al.*, (2014) recorded different response of chickpea genotypes to rhizobium inoculation, many genotypes were recorded with medium to high response, some showed low to medium increase and a small number of genotypes were observed with no response or somewhat decrease in nodules and seed yield g plant⁻¹. The possible explanation of differences in response of miscellaneous genotypes to rhizobium inoculation could be the compatibility and interaction between rhizobium and genotypes. Romdhaneet *al.* (2007) also found variable response to inoculation in different chickpea cultivars. Moreover, Katy (2009) reported that the benefits of symbiosis with rhizobia for *Medicago truncatula* ranged from uncooperative to highly mutualistic. He also reported significant genotype by rhizobial strain interaction for yield and yield components and concluded that, the performance of rhizobia strain is significantly modified by the plant genotype, the strain which was highly effective in one cultivar could be rated moderately or less efficient in other cultivar. Reports of Otienoet *al.* (2009) also indicated the variable influence of inoculation on grain legumes that depends on species, parameter being measured and other environmental factors. The above mentioned observations identified the importance of considering both symbiotic partners while attempting to improve different plant parameters.

Seed yield g plant⁻¹ correlated positively significantly with nodule dry weight plant⁻¹, nodule wet weight plant⁻¹, number of filled pods plant⁻¹ and number of seeds plant⁻¹. However, Bhuiyanet *al.* (2008) observed non-significant correlation between seed yield plant⁻¹ and nodules plant⁻¹ in chickpea. These traits could be accepted criterion for selection of the most effective legume- *Rhizobium* associations.

CONCLUSION

- From the present study it was concluded that breeding efforts are needed to develop chickpea genotypes having positive and highly significant association with rhizobium strains that found in our soils and produce nodules consistently across a wide range of chickpea growing regions in Sudan to overcome problem of nitrogen deficiency.
- Nodulation and seed yield of chickpea can be improved by inoculation with compatible rhizobia which could be

economically feasible to increase chickpea production.

- Develop chickpea genotypes having positive and highly significant association with rhizobium strains that are inoculated.
- Salwa cultivar showed high nodulating compare to Burgeig cultivar so it could be used successfully in crop rotation programs after result confirmation.

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