Impacts of *Rhizobium* Strain Ar02 on the Nodulation, Growth, Nitrogen (N_2) Fixation Rate and ion Accumulation in *Phaseolus vulgaris* L. under Salt Stress

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10.18805/LRF-4716

ABSTRACT

Background: *Phaseolus vulgaris* L.-rhizobia symbiosis has effectively enhanced common bean productivity *via* multiple biological mechanisms. This study aims to assess the impacts of the strain of *Rhizobium* on the nodulation, growth, nitrogen (N_2) fixation rate and ion accumulation within *Phaseolus vulgaris* L. under salt stress.

Methods: The Coco Blanc cultivar of the common bean was inoculated with the Ar02 rhizobia strain at 15 days after germination. Bean plants were inoculated in perlite culture to which salt was added in concentrations of 0, 25, 50 and 75 mmol L^{-1} NaCl.

Result: Inoculation with the Ar02 rhizobia strain led to infective and effective symbiosis with the common bean plants exposed to saline solutions and non-saline solutions, respectively. Nodule biomass and nitrogen content declined under salt stress but maintained a higher number of nodules and nodule biomass at 75 mM NaCl. Plant root and shoot length increased with higher biomass under saline conditions, significantly more than the non-inoculated plant without salt. However, the progressive addition of NaCl reduced the growth of the root and shoot and the biomass within the inoculated plant. Salinity led to increased Na^+ within the plant's shoot, along with a reduction in Ca^{+2} and K^+ concentrations. The shoot's Ca^{+2}, Na^+ and K^+ content were higher in the inoculated plant than the non-inoculated. The salt tolerance in common bean plants inoculated with Ar02 rhizobia was linked with the plant's capability to sustain nodulation and enhance Na^+ concentration in the shoot. Furthermore, salt tolerance within the same variety inoculated with *Rhizobium* was linked to a decline in the Ca^+ and K^+ concentrations in the shoot region of salt-exposed plants.

Key words: Ion accumulation, N_2 fixation, *Phaseolus vulgaris* L., Rhizobia, Salinity.

INTRODUCTION

One of the most critical factors threatening agricultural productivity worldwide is soil salinisation (Morgan *et al.,* 2018; Dutta and Bera, 2014). According to El-Akhal *et al.* (2013), salinisation affects approximately one-third of the 260 million ha dedicated for agricultural irrigation.

Legumes are highly beneficial plants with high grain protein concentrations capable of biological nitrogen fixation (Khan, 2018). Due to high similarity, legumes are categorised as salt-sensitive crops. However, an increase in soil salt concentration could adversely affect the microbial population found within the soil, commonly called rhizobia, which forms symbiotic relationships with the legumes (Rosas *et al.,* 2009). Furthermore, saline sensitivity becomes more complicated in legume nodulation and during symbiotic *Rhizobium*-complex interactions (Benidire *et al.,* 2017). High salt tolerance has been observed *in vitro* (Singleton *et al.*, 1982) in comparison to the respective host regimes, along with improved *Rhizobium* survival and growth under the influence of high osmotic pressure. Nevertheless, the characteristics of different *Rhizobium* species and strains vary, further influencing the salt tolerance level (Mohammad *et al.,* 1989).

Salinity can be categorised as abiotic stress, which influences plant productivity. Under the influence of the osmotic effect, salinity leads to stomatal closure, diminishes CO_2 fixation and disrupts the CO_2/O_2 ratio within the leaves (Ahmad *et al.,* 2011). Salt negatively impacts the plant when it accumulates within the plant tissues (Ben Khaled *et al.,* 2003). Nonetheless, salinity-induced reduction of plant growth is not linked to Cl^- and Na^+ toxicity (Hu *et al.,* 2005). According to Chen *et al.* (2005), salt treatment efficacy is connected to the stimulation of the K^+ efflux and the depletion of cellular K^+ pools and depends on the sensitivity of the specific cultivar to salinity. Hypersensitivity is negatively correlated with the plant's capability to absorb K^+, as

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How to cite this article: Kouki, S., L'taief, B., Al-Qthanin, R.N. and Sifi, B. (2021). Impacts of *Rhizobium* Strain Ar02 on the Nodulation, Growth, Nitrogen (N_2) Fixation Rate and ion Accumulation in *Phaseolus vulgaris* L. under Salt Stress. Legume Research. 44(12): 1529-1533. DOI:10.18805/LRF-4716.

Submitted: 01-07-2021 **Accepted:** 20-08-2021 **Online:** 22-09-2021

observed in *Arabidopsis* mutants and tomatoes (Borsani *et al.,* 2001). *Arabidopsis* growth is limited in the presence of K^+ and Ca^{2+}, specifically when NaCl and these two ions are present in the root region; however, the results differ when the roots are placed in a control medium (Attia *et al.,* 2008).

Additional investigation is needed on the potential influence of the legume-*Rhizobium* strain combination on symbiotic N-fixation grown under salt stress. In addition, further examination of plant activities via the use of tracers and accumulated ions is needed (Waisel*,* 1989). In this study, we investigate the impacts of the specific *Rhizobium* strain on the nodulation, growth, nitrogen (N_2) fixation rate and the accumulation of ions within the common beans plants growing under salt stress.

MATERIALS AND METHODS

Experimental treatments and biological material

The Coco Blanc variety of *Phaseolus vulgaris* L. (The common bean) was selected for this study as it is commonly grown in Tunisia. A 6.7% solution of $Ca(ClO)_2$ was used to sterilise the common beans for 15 min, after which four volumes of sterile distilled water were used to wash the seeds carefully. Seed germination required three days in Petri dishes covered by a layer of sterile moistened blotting paper. The common bean seeds and the locally grown Ar02 rhizobia were provided by the Laboratory of Sciences and Techniques Agronomics, National Institute of Agronomic Research in Tunisia (INRAT, Tunisia). The liquid culture of rhizobial inoculants was prepared in YEM medium (Vincent, 1970) that was applied by wetting the seedlings for 30 min within the inoculants before transplantation into plastic growth pots containing 0.5 kg sterile perlite. The transplants were grown in the greenhouse. NaCl concentrations were 0, 25, 50 and 75 mM. There were a total of eight treatments and every treatment was repeated four times. Pre-germinated seeds were sown in pots containing sterile perlite and assigned to the following treatments: T1- 0 mM NaCl, T2- 0 mM NaCl + Ar02, T3- 25 mM NaCl, T4- 25 mM NaCl + Ar02, T5- 50 mM NaCl, T6- 50 mM NaCl + Ar02, T7- 75 mM NaCl and T8- 75 mM NaCl + Ar02. Irrigation was performed twice a week using a nutrient solution (40 ml/pot) without nitrogen (Vincent, 1970). The following growth parameters were measured: nodulation bearing on the nodule number and biomass, growth (shoot and root length) and shoot and root biomass were determined in the flowering stage.

Data analysis and harvest

Plants were harvested during the early flowering stage. Nodules were removed from the roots while the plants were segregated from the roots and shoots and oven-dried at 70°C for 72 h. After measuring the dried weights, sample shoots were individually ground and nitrogen content was measured utilising the Kjeldahl procedure. Cation elements Ca^{2+}, Na^+ and K^+, were quantified by atomic absorption spectrophotometry (Spectrometer series). Calibration was performed for each cation, using 1 g L^{-1} standards (1 mL injected) prepared in the same manner as the samples. The dry weights of roots and shoots of individual plants were measured and the symbiotic parameters measured were dry nodule weight and nodule number, length of root and shoot and %N, %K^+, %Na^+ and %Ca^{2+} content.

Statistical analysis

The randomised complete block was selected as the experimental design. For evaluation through the statistical analysis, SPSS 20 software was used. Data were analysed using ANOVA and means were compared using the Fisher's LSD test. Statistical significance was assigned at $p<0.05$.

RESULTS AND DISCUSSION

As seen in Fig 1A and B, in general, as salinity increased, biomass and the number of nodules declined. However, there were higher biomass and nodule numbers at 75 mM NaCl, indicating that the Ar02 strain of *Rhizobium* had a higher tolerance for higher than moderate salinity. These results are different from those obtained in experiments with chick-pea plants, in which 50 mM NaCl concentration led to a 60% decline in nodulation (Elsheikh and Wood, 1990). Furthermore, chick-pea plant nodulation under exposure to the highest salinity concentration was completely depressed after four weeks. Hafeez *et al*. (1988) revealed a 50% nodulation reduction when *Vigna radiata* plants (mung beans) were exposed to plants with salinity >50 mM. Reduction in nodule formation at low salinity levels could result from a strong salinity influence on the initiation of the nodulation process. Nodule initiation is an event within *Rhizobium*-legume symbiosis and demonstrates high sensitivity to osmotic stress. According to Bhardwaj (1975), a higher salinity tolerance was demonstrated by *Bradyrhizobium* and *Rhizobium* than the legume host, indicating successful symbiosis depending on the level of tolerance of the host plants.

Root and shoot growth and biomass increased in Ar02 rhizobia-inoculated plants (with and without added salt) compared to non-inoculated plants. A gradual reduction in the root and shoot growth and biomass was detected with NaCl addition within the inoculated plants; however, the growth of root and shoot and biomass increased in the 50 mM NaCl inoculated plants. Similarly, salinity led to a decline in the weight of shoots and roots within the plants of the legumes green bean (*Phaseolus vulgaris*) (Gulmezoglu, 2016), soybean (*Glycine max*) (Grattan and Maas, 1988) and faba bean (Yousef and Sprent, 1983). Cowpea plants (*Vigna radiata*) survived (matured) for up to 65 days at 90 mM NaCl (Hafeez *et al.,* 1988), whereas chickpea plants (*Cicer arietinum*) lived for 30 days (Elsheikh and Wood, 1990).

Increases in the roots and shoots of the common beans inoculated with Ar02 rhizobia strain and exposed to salinity are shown in Fig 1C-F with the results of non-inoculated plants. As seen in Table 1, there was no improvement in the overall content of shoot nitrogen to affect inoculation at different salt concentrations. With an extreme decline in the nodule number and biomass, the results of this study indicate that common beans inoculated with rhizobia Ar02 grew under salinity, which led to the accumulation of limited N_2 in shoots. The findings contrast with Singleton (1983) and Weil and Khalil (1986), who detected N_2 concentration declines.

Na^+ shoot content increased significantly as the salt dose increased with and without inoculation. As seen in Table 1, increased Na^+ did not significantly hinder plant growth in the shoots of plants inoculated with Ar02 compared to the

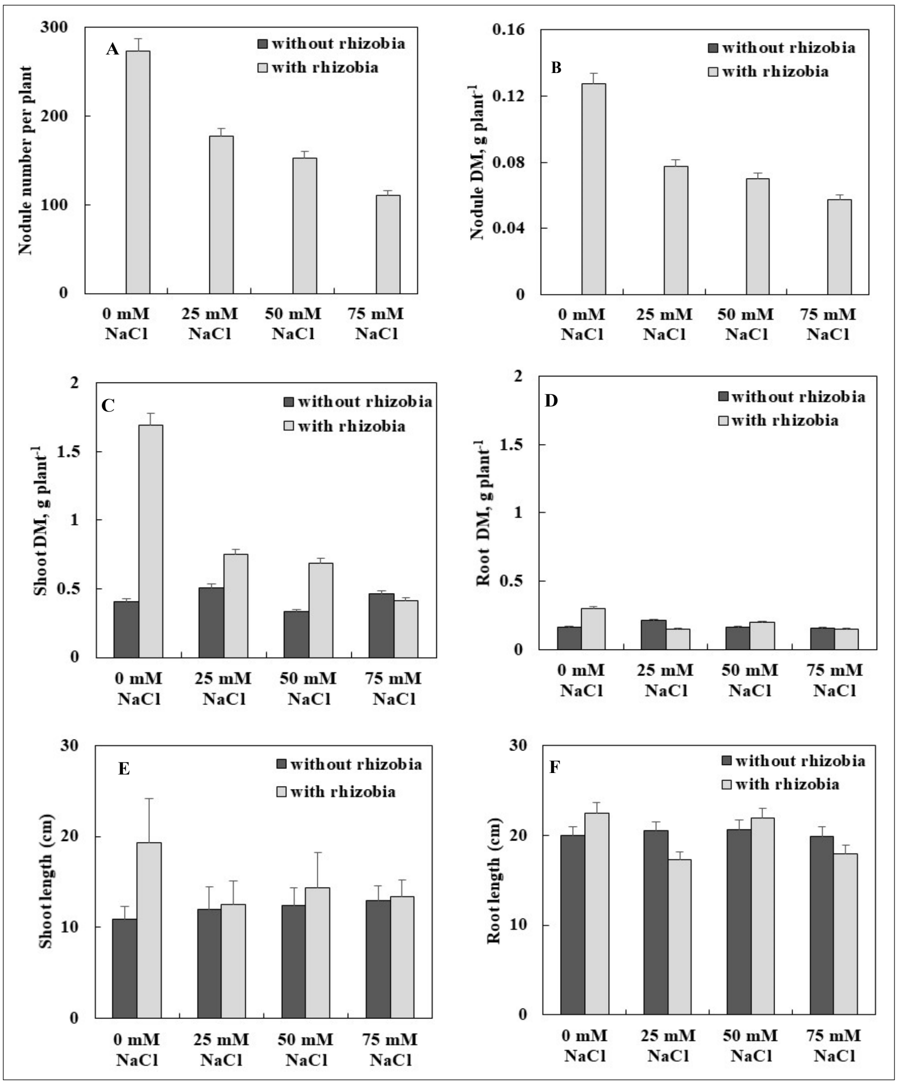

Fig 1: Effect of NaCl (0, 25, 50, 75 mM) on nodule number (A), nodule biomass (B) shoot biomass (C), root biomass (D), shoots lenght (E) and roots lenght (F) of common beans. Variety: Coco Blanc. NaCl (0, 25, 50, 75 mM) was added after 15 days of growth. Plants were harvested 45 DAS (the day after sowing). Data are the means±SD of four replicates.

Table 1: Effect of NaCl (0, 25, 50, 75 mM) on nitrogen (N), sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) contents (%) in shoots of common beans.

	Without rhizobia				With rhizobia			
	0 mM NaCl	25 mM NaCl	50 mM NaCl	75 mM NaCl	0 mM NaCl	25 mM NaCl	50 mM NaCl	75 mM NaCl
% N	2.20±0.07	2.10±0.01	2.04±0.08	1.99±0.00	2.80±0.02	1.91±0.24	1.85±0.22	1.66±0.10
% K^+	1.80±0.01	1.48±0.35	0.76±0.01	1.15±0.01	1.98±0.01	1.52±0.37	1.54±0.37	1.32±0.35
% Na^+	0.36±0.00	0.58±0.08	0.73±0.01	0.88±0.03	0.62±0.01	0.66±0.01	0.86±0.02	0.94±0.01
% Ca^{2+}	0.70±0.00	0.88±0.15	0.65±0.00	0.91±0.00	1.10±0.00	1.04±0.009	0.83±0.005	0.60±0.15

Variety: Coco Blanc. NaCl (0, 25, 50, 75 mM) was added after 15 days of growth. Plants were harvested 45 DAS (the day after sowing). Data are the means ±SD of four replicates.

plants not challenged with salinity (Table 1). It is very difficult to alleviate the adverse impacts of salt in hydro-aeroponic cultures, specifically in salt-tolerant species. Our results indicate absorbed Na^+ ions significantly impacted growth.

The uptake of Ca^{2+} and K^+ was adversely influenced by salinity, as shown in Table 1, which is consistent with the results of Ushakova *et al.* (2005) and L'taief *et al.* (2020). Competition between K^+ and Na^+ at the absorption sites influence K^+ intake through the common transport system (Khan, 2000). There is high selectivity for K^+ within the transport system, which assists in Na^+ leakage and any drastic decline in the accumulation of K^+ in response to varying halophytes. A decrease in the efficiency of K^+ uptake in plants resulted from direct competition within root transporters to uptake K^+ and Na^+. Due to similarity in the physiochemical properties of Na^+ and K^+, higher Na^+ concentrations lead to reduced K^+ uptake by the roots.

CONCLUSION

The tolerance to salinity stress in common beans (Coco Blanc variety) inoculated with rhizobial strain Ar02 was characterised by excluding Na^+ from the aerial portion of the plant. The nodulation process of common beans was exposed to ≤5 mM NaCl or less and the growth of common bean plants was significantly improved. In addition, the salt tolerance of this bean variety inoculated with *Rhizobium* was associated with a decrease in the concentration of K^+ and Ca^{2+} in shoots. Microorganisms found within the soil are closely interconnected with plant roots, thereby playing a critical role in plant growth under stress. These findings requiring agronomic validation depict that the impact of inoculation in enhancing the growth of the aerial plants, nodulation and the Na^+ concentration in the salt-stress conditions.

ACKNOWLEDGEMENT

The Ministry of Higher Education and Scientific Research and the Ministry of Agriculture in Tunisia provided financial support for this work.

Conflicts of interest

The authors have declared no conflicts of interest.

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