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Interaction between faba bean cultivars and the *Rhizobium leguminosarum* strains: Symbiotic N_2 fixation and protein profiles under salt stress

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The efficiency of eight *Rhizobium leguminosarum* strains isolated from root nodules of Faba bean plants which were collected from different Governorate in Egypt has been examined. After inoculation of faba bean seeds with each *Rhizobium* strain individually, we measured the growth promotion, nodule formation and plant protein profile under different concentration of sodium chloride salt stress. The interactions between salt tolerant and sensitive faba bean cultivars and *Rhizobium* strains under NaCl stress were found to be significant. Nitrogen fixation efficiency varied among the eight *Rhizobium* strains. The protein profile using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for both faba bean cultivars revealed that *Rhizobium* strains numbers RL1, 2, 4 and RL 8 showed high level of salt tolerance under 40 and 80 mM NaCl in both faba bean cultivars. *Rhizobium* strains improved the salt tolerance, as is clearly observed in number of nodules and the amount of N_2 fixation. It is known that stress affects the growth, metabolic activity and symbiotic efficiency of *Rhizobium* with faba bean plant but in this study we found that *Rhizobium* strains numbers RL2 and RL 3 showed high efficiency of N_2 fixation and growth promotion in both faba bean cultivar.

Key words: Faba bean, *Rhizobium*, nodulation efficiency, salt stress, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protein profile.

INTRODUCTION

Symbiotic nitrogen fixation (SNF) by legumes plays an important role in reinforcing crop productivity and conserving the fertility of peripheral land and in the smallholder system of the semi-arid tropics. It is expected that the importance of legumes and symbiotic nitrogen fixation will continue to increase the development of national

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sus-tainable agriculture. The major step toward maximum SNF technology is the increment of land area under legumes and enhances their grains and fodder yield through overcoming environmental condition problems which limit SNF and legume productivity. However, SNF by legumes is particularly sensitive to environmental stress like drought, soil salinity, acidity and low phosphorus (Zahran, 1999). The most serious threat faced by agriculture in arid and semi-arid regions is salinity (Rao and Sharma, 1995); unsuccessful symbiosis under salt-stress may be due to failure in the infection process, because of the effect of salinity on the establishment of rhizobia (Fauvart and Michiels, 2008). The salt damage on the symbiotic interaction not only inhibits the formation of nodules, but also leads to the reduction of the growth of the host plant.

Other effects of salinity on the nodulation include formation of non-functional nodules with abnormal structure and degradation of peribacteroid membrane (Bolaños et al., 2003). Strategies to improve legume production in saline environments include selection of host genotypes that are tolerant to high salt conditions; inoculation with salt-tolerant strains of rhizobia could constitute another approach to improve legume productivity under symbiosis (Hassan and Eissa, 2013). The improving of the symbiotic efficiency and legume production under this constraint should target both symbiotic partners, together with appropriate crop and soil management (Fernandez et al., 2010; Sharma et al., 2013). Furthermore, Nearly 40% of the world land surface can be categorized as having potential salinity problems affecting soil fertility. Most of these areas are confined to the tropics and the Mediterranean regions. It is important to select more effective strains for specific cultivars from the region where common beans are cultivated intensively. Moreover, O'Hara et al. (2002) reported that the abundance of diversity in the soil populations of rhizobial strains provides a large resource of natural germplasm to screen desired characteristics present in the natural pool. Also, Workalemahu (2009) stated that the presence of diversity among the strains revealed the possibility of getting potentially effective adaptable *Rhizobium* strains that enhance faba bean productivity.

Little is known about salinity effect on nodule pattern distribution in different regions of the root system, and their efficacy to establish a symbiotic association that is, the ability of the *Rhizobium*-legume to develop a partnership for forming nodules. Although the root nodule colonization of *Rhizobium* bacteria are more salt tolerant than their legume hosts, they showed marked variation in salt tolerance. The present study examines the nodulation and nitrogen fixation efficiency of new *Rhizobium* strains under salt stress in faba bean plant to improve the crop productivity and sustaining the fertility of land in arid and semiarid regions.

MATERIALS AND METHODS

The study was conducted from 2011 to 2013.

Rhizobium leguminosarum strains

Eight *Rhizobium* strains were isolated from faba bean root nodules that were collected from different locations of Egyptian soil. These strains of *R. leguminosarum* were identified according to Shoukry et al. (2013). All strains were purified, characterized and identified as belonging to the genus *R. leguminosarum* bio*. viciae*. The purified strains have been tested for their nodulation and growth potential under salt conditions. Faba bean seeds were inoculated with *Rhizobium* strains to study their efficiency of nodulation by measuring number of nodule; plant height; plant fresh and dry weight; total nitrogen; phosphorus content; potassium and sodium content in faba bean plants under different concentration of sodium chloride NaCl (0; 10; 20; 40 and 80 mM) to be added in irrigation water.

Growth conditions of the *Rhizobium* strains and experimental pots

The selected strains were examined to grow in Yeast extract Mannitol (YEM) broth (Vincent, 1970) containing 0 to 80 mM NaCl (pH7.0) with three replicates in a gyratory shaker at 150 rpm and 30°C. Cells grown in YEM brothfor 18 h were used as an inoculums (10^9 C.F.U. /ml) and then sown in 42X60 cm pots (in three replicates), each containing 5 kg sandy soil. The soil in these pots was saturated by watering with saline water (1000 ml of saline water perpot) containing 0 to 80 mM NaCl. Inoculated plants were also maintained on water without NaCl as controls. The plants were irrigated with 300 ml Brogan's modified cronies solution which consists of g/L: KCl_2 10.09; $CaSO_4$-$2H_2O$ 2.5; $MgSO_4$-H_2O 2.5; Ca (PO_4) 2 2.5 and Fe PO_4 2.5 (Allen, 1961) nitrogen free with different concentration of NaCl: 0; 10; 20; 40 and 80 mM /pot/three days. The plants were removed after 60 days then number of nodules, plant height (cm), fresh weight (g) and dry weight (g) were recorded.

Salt stress

Tolerant and salt sensitive cultivars seeds of faba bean (Giza 843 and Sakha 1 cultivars) were used in this study obtained from Legumes Research Department Agronomy Research Institute, Agricultural Research Center, Giza, Egypt. The pot experiment was carried out at experimental farm Faculty of Agriculture Al- Azhar University, Nasr city, Cairo, Egypt.

N, P, K and Na determination

The plant samples were collected from each treatment randomly. Fresh and dry biomass of whole plant was recorded. Total nitrogen, sodium, phosphorus and potassium contents of treated faba bean plants were estimated in the plant digest according to the method described by Faithfull (2002).

SDS-protein electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli, (1970). Total soluble proteins of the whole plant powder fully were taken from two cultivars, Sakha1 and Giza823 after treatment with NaCl in large pot. Protein fractionations were performing exclusively on vertical slab (19.8 × 26.8 × 0.2 cm) gel using the electrophoresis apparatus manufactured by LABOCONCO. Gels were analyzing using Total Lab TL100.

Statistical analysis

The data were subjected to statistical analysis, employing F-test for significance ($P \leq 0.05$) and computing of "Least Significant Difference (L.S.D.)" values to separate means in different statistical groups as described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Number of nodules/plant

There was a significant difference ($P \leq 0.05$) in the number of nodules per plant between different *Rhizobium* strains. However, data in Tables 1 and 2 showed that the strain RL1 revealed the highest values for number of nodules above all strains under different concentrations of NaCl in the two faba bean cultivars (Giza 843 and Sakha 1). The average number of nodules for RL1 strain was 198.37 nodules per plant at control treatment and decreased to 62.59 nodules per plant at 80 mM NaCl concentration in Giza 843 cultivar while, RL1 strain had 175 nodules per plant at control treatment and 36.33 nodules per plant at 80 mM NaCl concentration in Shaka1 cultivar. The negative effect of a high level of salinity on number of nodules per plant were also found by Fetyan and Mansour (2012), who reported that high salt concentration inhibited the growth of the three *Rhizobium* strains. Furthermore, Younesi et al. (2013) reported that nodulation was completely inhibited under salt stress condition. In this respect, Fahmi et al. (2011) reported that the increase of seawater concentration (salinity level) decreased the average number of nodule through nodule formation by the inhibition of initial steps of *Rhizobium*-legume symbioses. In this concern, the variation in nodulation could be due to low rhizobial density, incompatibility of the rhizobia and edaphic factors that hinder the effecttiveness of the rhizobia. Moreover, Fetyan and Mansour (2012) stated that the limitation of symbiosis under saline conditions may be due to: (i) survival and proliferation of *Rhizobium* spp. in the soil and rhizosphere; (ii) inhibiting the infection process, (iii) directly affecting root nodule function, or (iv) reducing plant growth, photosynthesis, and demand for nitrogen.

On other hand, Belal et al. (2013) showed that the *R. leguminosarum* bio*. viciae* isolated from different geographic regions soils are able to survive, grow and effecttively nodulated on faba bean even at high salt concentrations.

Plant height (cm)

Significant differences ($P \leq 0.05$) were observed between eight *Rhizobium* strains under different salt concentrations on plant height (Tables 1 and 2). The results reveal that the tallest plants (63.56 and 61.22 cm) were obtained in plants inoculated with RL6 and RL7 strains under control conditions; the same two strains had given the tallest plants (50 and 46.63 cm) under 80 mM NaCl concentration in Shaka1 cultivar. Conversely, the tallest plants (68 and 66.67 cm) were obtained in plants treated with RL5 and RL4 strains under 0 mM NaCl (control) while, under 80 mM NaCl concentration the tallest plants (56.49 and 56 cm) were obtained in plants treated with RL1 and RL8 strains in Giza 843. The variation among treated plants under salt stress and control in plant height may be due to the harmful effects of salinity on availability of nutrients for growth of plants. Supporting results were obtained by Al-Shaharani and Shetta (2011) who indicated that salt stress caused a significant depression in seedling growth parameters (seedling height) of both studied *Acacia* species and it seemed to reduce the availability of the nutrients required for the growth and then development of the plants comparing with the control.

Fresh weight (g/plant)

Fresh weights of faba bean plants treated with different *Rhizobium* strains were significantly affected at the 5% level. However, careful observations of data in Tables 1 and 2 clearly show that plants inoculated with RL7 and RL2 strains were superior in fresh weight per plant under all NaCl concentrations in Giza 843 and had 185.55, 183.67, 145 and 108.33 g/plant under control and 80 mM NaCl respectively. On contrast, the maximum amounts of fresh weight per plant (164.54 and 159.07 g/plant) were recorded in plants inoculated with RL4 and RL2 strains, respectively, under control condition. While the maximum amounts of fresh weight per plant (96.22 and 86.33 g/plant) were recorded in plants treated with RL5 and RL6 strains respectively under 80 mM NaCl. This obtained result goes in line with those findings by Alshammary et al. (2004) who reported that salinity reduces shoot and root weights in several legumes. In addition, increasing the salinity level of solution reduced the fresh and dry weights of Savory plant. However, Öğütçü et al. (2010) concluded that salinity levels negatively affected all parameters of growth such as root and shoot growth in chickpea plants. The decrease in plant biomass production due to salinity may be attributed to low or medium water potential, specific ion toxicity, or ion imbalance.

Dry Weight (g/plant)

There were significant differences ($P \leq 0.05$) in the dry weight between different *Rhizobium* strains as shown in Tables (1 and 2). However, the maximum amounts of dry weight per plant (28.67 and 27.51 g/plant) were recorded in plants inoculated with RL2 strain under 20 mM of NaCl and plants inoculated with RL7 strain under control respectively without significant differences between them. Moreover, plants inoculated with RL7, RL6 and RL2 strains gave the maximum amounts of dry weight per plant (15.85, 15.48 and 14.67 g/plant) with significant differences between them and control in Giza 843 cultivar. Regarding Sakha 1, the maximum amounts of

Table 1. Effect of salinity on number of nodules, plant height, fresh and dry weights of faba bean cultivar Giza 843 inoculated with different *Rhizobium* strains.

***Rhizobium* strains**	**Salinity levels (mM)**				
	0	**10**	**20**	**40**	**80**
Number of Nodules / Plant					
RL1	198.37	157.67	127.68	114.70	62.59
RL2	126.77	116.18	125.00	76.33	35.67
RL3	41.33	38.67	33.33	22.67	11.78
RL4	69.67	68.67	57.33	41.67	27.22
RL5	93.21	81.33	79.11	55.66	25.67
RL6	137.67	136.33	109.00	67.67	39.67
RL7	135.33	127.67	93.67	61.48	27.67
RL8	146.37	158.33	96.65	79.52	64.67
LSD at 5% = 8.13					
Plant Height (cm)					
RL1	54.89	54.62	49.83	43.16	56.49
RL2	55.33	53.34	54.21	54.07	48.67
RL3	56.21	52.33	50.67	47.33	46.00
RL4	66.67	65.67	51.33	48.22	45.33
RL5	68.00	58.67	47.33	46.11	44.67
RL6	59.71	52.33	52.47	48.67	44.67
RL7	59.85	56.00	53.67	51.00	46.00
RL8	49.81	47.67	49.00	42.00	56.00
LSD at 5% = 7.99					
Fresh Weight (g/plant)					
RL1	129.87	119.04	103.10	92.67	63.48
RL2	183.67	179.00	152.00	133.00	108.33
RL3	127.00	82.11	81.33	68.67	63.00
RL4	179.33	176.00	164.33	104.11	98.53
RL5	179.22	165.89	156.33	123.43	108.52
RL6	163.52	158.00	140.00	120.00	106.00
RL7	185.55	178.00	163.00	160.00	145.00
RL8	148.25	146.00	143.00	134.00	91.00
LSD at 5% = 7.89					
Dry Weight (g/plant)					
RL1	17.88	17.88	14.15	11.27	9.55
RL2	27.33	28.67	23.33	18.33	14.67
RL3	18.92	13.56	11.54	9.33	6.63
RL4	26.12	22.55	18.95	16.74	14.52
RL5	19.55	18.95	16.18	15.77	14.18
RL6	18.92	17.87	16.77	15.49	15.48
RL7	27.51	27.21	24.32	19.89	15.85
RL8	18.67	15.00	13.22	12.00	9.85
LSD at 5% = 4.34					

dry weight per plant (24.48 and 22.03 g/plant) were recorded in plants inoculated with RL2 and RL4 strains under control. Furthermore, plants inoculated with RL5, RL6 and RL7 strains gave the maximum amounts of dry weight per plant (12.16, 10.22 and 10.04 g/plant) with significant differences between them and control. Peoples

Table 2. Effect of salinity on number of nodules, plant height, fresh and dry weights of faba bean cultivar Shaka 1 inoculated with different *Rhizobium* strains.

***Rhizobium* strains**	**Salinity Levels (mM)**				
	0	**10**	**20**	**40**	**80**
Number of nodules / Plant					
RL1	175.00	135.00	113.00	78.33	36.33
RL2	115.41	76.53	63.34	61.33	27.74
RL3	21.78	19.17	10.32	7.89	5.10
RL4	66.55	62.93	37.57	28.94	17.20
RL5	78.62	69.33	59.33	50.40	23.22
RL6	114.91	107.40	97.33	38.67	27.00
RL7	107.33	66.67	55.33	34.33	19.67
RL8	77.56	81.00	41.33	38.00	25.00
LSD at 5% = 5.97					
Plant height (cm)					
RL1	59.00	53.67	53.33	45.33	40.33
RL2	57.85	56.22	53.26	46.88	43.37
RL3	54.91	53.49	42.03	41.18	40.29
RL4	59.87	56.88	51.03	43.62	38.15
RL5	49.52	48.00	46.37	44.66	41.33
RL6	63.56	59.65	50.67	48.33	46.63
RL7	61.22	53.33	59.00	51.00	50.00
RL8	56.33	50.60	55.32	52.68	45.00
LSD at 5% = 7.03					
Fresh weight (g/plant)					
RL1	85.33	76.00	76.33	45.67	28.00
RL2	159.07	153.40	114.07	100.21	85.01
RL3	95.85	74.59	66.41	60.41	62.47
RL4	164.54	162.51	151.16	98.63	77.77
RL5	152.19	144.89	126.33	109.55	96.22
RL6	139.00	125.00	115.00	109.00	86.33
RL7	125.67	127.00	113.07	102.02	76.00
RL8	108.52	100.00	90.00	83.00	76.00
LSD at 5% = 7.85					
Dry weight (g/plant)					
RL1	16.85	14.11	11.27	9.62	7.26
RL2	24.48	16.57	12.29	11.91	9.52
RL3	17.92	8.59	7.38	7.70	6.78
RL4	22.03	20.20	15.81	11.69	8.74
RL5	17.17	16.51	15.94	12.51	10.22
RL6	14.52	16.52	13.87	13.57	12.16
RL7	15.96	14.23	13.54	11.23	10.04
RL8	15.55	14.37	12.32	9.62	5.95
LSD at 5% = 3.82					

et al. (2002) explained that shoot dry matter is a good indicator of relative isolate effectiveness. However, Al-Shaharani and Shetta (2011) indicated that salt stress caused a significant depression in seedling growth parameters (dry weight) in both *Acacia* species it seemed to reduce the availability of the nutrients required for the growth and then development of the plants comparing with the control. Belal et al. (2013) found that *R. leguminosarum* bv *viciae* improved the growth parameters dry weight of shoot of faba bean plants while, Mnalku et al. (2009) found that significant correlation between nodule number and nodule dry weight with shoot dry weight substantiates the N fixation efficiency of strains (nodules).

Nitrogen percentage

Nitrogen is an essential and often limiting plant nutrient in crop production. Data tabulated in Tables 3 and 4 showed that, significant differences at the 5% level among different applied *Rhizobium* strains. However, total nitrogen content accumulated in faba bean plants inoculated with RL3, RL4 and RL1 strains had showed more nitrogen content above all strains at different NaCl concentrations in Giza 843 while, faba bean plants inoculated with RL5, RL2 and RL8 strains had showed more nitrogen content above all strains at different NaCl concentrations in Shaka1 cultivar. The maximum nitrogen percentage (4.09%) were noticed in plants treated with RL3 strain in Giza 843 cultivar at 80 mM NaCl followed by plants treated with RL5 strain which had given 4.92% at 40 mM NaCl in Shaka1 cultivar. These results are in agreement with those obtained by Al-Fredan (2006) indicate that locally isolated strains of *R. leguminosarurn* inoculated faba bean plants fixes much greater N, than when introduced strain are used. Also, salinity tolerant rhizobial strains increased significantly the total N2 mg/plant in saline sandy soil compared with the inoculation with salinity un-tolerant rhizobial isolates. However, the harmful effects of salinity on Nitrogen fixing or content were obtained by many authors (Zahran, 2001; Zheng et al., 2009; Al-Shaharani and Shetta, 2011). The reasons for decline nitrogen content under salt stress that chlorine ions limit the absorption of N_2. Antagonistic effect between Cl and N_2 is well known in glycophytes than halophytes plants; the latter are able to absorb efficiency N_2-even under high salt condition. Moreover, salt-induced distortion in nodule structure could also be a reason for the decline in the N_2 fixation rate by legumes subjected to salt stress. In addition, the reduction of N_2-fixing activity by salt stress usually attributed to a reduction in respiration of the nodules (Kenenil et al*.* 2010).

Phosphorus percentage

Overall results show significant differences at the 5% level among different *Rhizobium* strains applied. However, data in Tables 3 and 4 revealed that, the highest percentages of phosphorus attained in faba bean plants inoculated with RL3 and RL2 strains were 0.30 and 0.21% at 40 mM NaCl and control treatments respectively, in Giza 843 cultiver whereas, the highest percentages of phosphorus attained in faba bean plants inoculated with RL5, RL2 and RL3 strains were 0.25, 0.23 and 0.23% at 80, 20 and 40 mM NaCl treatments respectively, in Shaka1 cultivar. Salt stress markedly decreased the plant P content in both alfalfa cultivars. Therefore, it can be stated that Salinity may be limiting factor for efficient nodulation and nitrogen fixation by decreasing the P content in the plants (Younesi et al. 2013).

Potassium percentage

Potassium percentage differed significantly ($P \leq 0.05$) between various treated faba bean plants with eight *Rhizobium* strains. However, data in Tables 3 and 4 showed that, the *Rhizobium* strains RL1 and RL2 are superior strains that had given maximum potassium percentages at different salinity levels in Giza 843 and Shaka1 cultivars respectively. Moreover, maximum potassium percentages 3.09 and 2.80 were recorded in plants inoculated with RL1 and RL5 at 80 and 20 mM NaCl in Giza 843 and Shaka1 cultivars respectively. These results were in line with those findings by Younesi et al. (2013) who reported that applying bacterial treatment, especially inoculation by *Rhizobium* strains were significantly increased the K^+ content of both alfalfa cultivars under salt stress conditions. Increased K concentration under saline conditions may help to decrease Na uptake and this can indirectly maintain the growth of the plant (Giri and Mukerji, 2004).

Sodium percentage

Results in Tables 3 and 4 indicate that, treated faba bean plants with different *Rhizobium* strains had effect on sodium percentage. However, careful observations of data clearly showed that, the highest sodium percentages 0.57, 0.52 and 0.51% were recorded in plants treated with RL3, RL1 and RL5 at control, 20 and 80 mM NaCl treatments in Giza 843cultivar. Conversely, the highest sodium percentages 0.59, 0.53 and 0.52% were recorded in plants treated with RL6, RL2 and RL8 at control, 80 and 40 mM NaCl treatments in Shaka1 cultivar. The *Rhizobium* and *Pseudomonas* can modify salt stress in alfalfa by increasing the total uptake of K, P, N and decreasing Na+ accumulation as compared to control. Also, accumulation of Na was strongly influenced by storage of other cations, particularly K (Younesi et al., 2013).

Faba bean Vegetative storage protein under salt stress inoculated with different strains of *Rhizobium*

The interaction between eight different *Rhizobium* strains with two faba bean varieties sensitive (Sakha1) and tolerant to salt stress (Giza 843) under different concentration of NaCl (10, 20, 40, 80 mM) was investigated using SDS-PAGE as illustrated in Figure 1. The analyzed vegetative storage protein profile indicates the nitrogen fixation

Table 3. Effect of salinity levels on nitrogen, phosphorus, potassium and sodium percentages of faba bean cultivar Giza 843 inoculated with different *Rhizobium* strains.

***Rhizobium* strains**	**Salinity Levels (mM)**				
	10	**20**	**40**	**80**	**Mean**
Nitrogen percentage					
RL1	1.02	1.44	1.44	1.53	1.36
RL2	1.50	1.34	1.21	1.02	1.27
RL3	1.09	1.47	1.85	4.09	2.13
RL4	1.53	1.12	1.66	1.21	1.38
RL5	1.02	1.09	1.31	1.02	1.11
RL6	1.12	1.44	1.21	1.44	1.30
RL7	1.40	1.15	1.21	1.40	1.29
RL8	1.28	1.37	1.12	1.15	1.23
Mean	1.25	1.30	1.38	1.61	
Phosphorus percentage					
RL1	0.10	0.03	0.07	0.14	0.09
RL2	0.21	0.16	0.01	0.07	0.11
RL3	0.07	0.14	0.30	0.12	0.16
RL4	0.13	0.03	0.05	0.14	0.09
RL5	0.09	0.05	0.11	0.03	0.07
RL6	0.13	0.08	0.04	0.08	0.08
RL7	0.15	0.05	0.07	0.11	0.10
RL8	0.07	0.08	0.05	0.11	0.08
Mean	**0.12**	**0.08**	**0.09**	**0.10**	
Potassium percentage					
RL1	1.59	1.98	2.15	3.09	2.20
RL2	0.19	1.50	1.05	0.99	0.93
RL3	0.89	1.08	1.08	1.51	1.14
RL4	1.83	0.94	1.22	1.49	1.37
RL5	1.66	1.36	2.07	1.65	1.69
RL6	1.23	1.50	0.85	1.29	1.22
RL7	1.31	1.85	1.41	1.40	1.49
RL8	1.56	1.30	1.62	2.13	1.65
Mean	**1.28**	**1.44**	**1.43**	**1.69**	
Sodium percentage					
RL1	0.37	0.52	0.43	0.33	0.41
RL2	0.21	0.49	0.21	0.30	0.30
RL3	0.57	0.32	0.36	0.31	0.39
RL4	0.43	0.45	0.29	0.46	0.41
RL5	0.37	0.40	0.40	0.51	0.42
RL6	0.22	0.31	0.18	0.43	0.29
RL7	0.30	0.30	0.39	0.30	0.32
RL8	0.41	0.40	0.44	0.43	0.42
Mean	0.36	0.40	0.34	0.38	

efficiency of the Rhizobium strains under salt stress using SDS-PAGE. Our results reveal various responses of *Rhizobium* strains for quantitative and qualitative protein bands in certain plant age and type under NaCl treatment. However, the low concentration of NaCl 10 and 20 mM does not affect the quantitative profile band compared to the control in both faba bean cultivars with all *Rhizobium* strains. Four *Rhizobium* strains (RL4, 5, 7 and 8) showed

Table 4. Effect of salinity levels on nitrogen, phosphorus, potassium and sodium percentages of faba bean cultivar Shaka1 inoculated with different *Rhizobium* strains.

***Rhizobium* strains**	**Salinity Levels (mM)**				
	10	**20**	**40**	**80**	**Mean**
Nitrogen Percentage					
RL1	2.17	1.72	2.46	1.66	2.00
RL2	3.58	1.92	2.78	2.81	2.77
RL3	1.85	1.88	2.30	1.98	2.00
RL4	1.92	2.11	1.44	0.96	1.61
RL5	1.92	2.97	4.92	2.36	3.04
RL6	2.04	2.30	1.76	1.82	1.98
RL7	2.97	2.01	2.07	1.98	2.26
RL8	2.14	2.07	2.81	2.04	2.27
Mean	2.32	2.12	2.57	1.95	
Phosphorus percentage					
RL1	0.15	0.13	0.20	0.18	0.17
RL2	0.23	0.23	0.19	0.18	0.21
RL3	0.11	0.13	0.23	0.21	0.17
RL4	0.13	0.09	0.16	0.08	0.12
RL5	0.13	0.04	0.15	0.25	0.14
RL6	0.05	0.11	0.09	0.04	0.07
RL7	0.15	0.17	0.20	0.19	0.18
RL8	0.14	0.14	0.17	0.12	0.14
Mean	0.14	0.13	0.17	0.16	
Potassium percentage					
RL1	1.45	1.10	1.07	0.75	1.09
RL2	1.56	1.53	1.81	1.54	1.61
RL3	1.31	1.12	1.72	1.73	1.47
RL4	1.56	1.15	1.44	1.54	1.42
RL5	1.71	2.80	0.81	0.84	1.54
RL6	1.75	1.25	1.21	0.92	1.28
RL7	1.18	1.11	1.33	1.06	1.17
RL8	1.61	1.67	1.14	1.06	1.37
Mean	1.52	1.47	1.32	1.18	
Sodium percentage					
RL1	0.38	0.28	0.36	0.32	0.34
RL2	0.35	0.31	0.27	0.54	0.37
RL3	0.27	0.38	0.39	0.36	0.35
RL4	0.34	0.45	0.40	0.36	0.39
RL5	0.36	0.42	0.41	0.28	0.37
RL6	0.59	0.34	0.29	0.27	0.37
RL7	0.39	0.34	0.40	0.31	0.36
RL8	0.42	0.36	0.53	0.39	0.43
Mean	0.39	0.36	0.38	0.35	

salt stress adaptation in both faba bean cultivars. They accumulate more band quantity gradually from 10 to 40 mM NaCl and then dramatically decreased in 80 mM NaCl concentration while, other strains (RL2, 3 and 6) showed increase of bands profile quantity from 10 to 80 mM NaCl. It was obvious to detect some unique bands (29 and

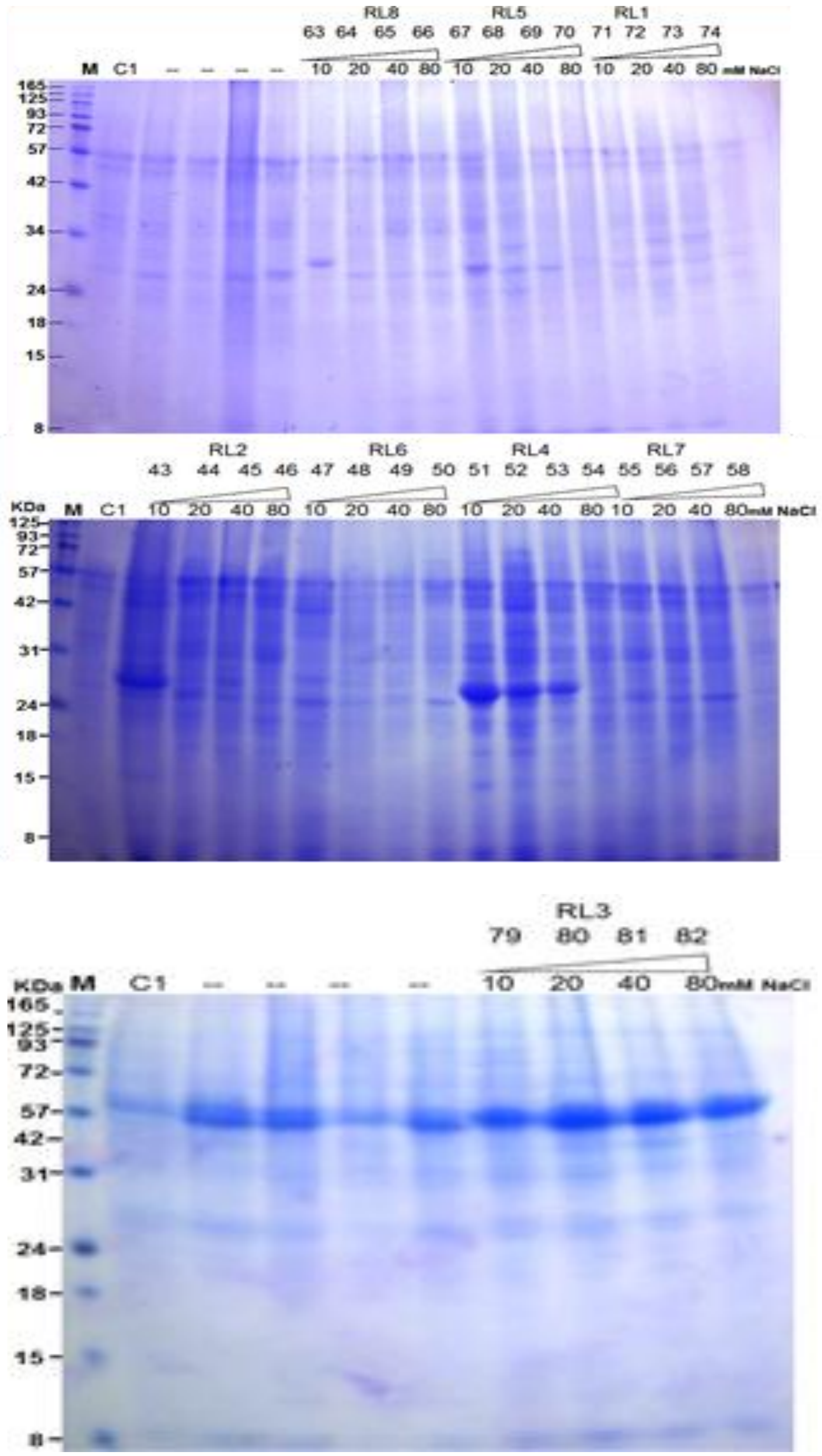

Figure 1. SDS-PAGE soluble protein profile of the interaction between Sakha 1 faba bean cultivar and the eight *Rhizobium* strains (RL 1, 2, 3, 4, 5, 6, 7 and 8) under four different concentration of NaCl (10, 20, 40 and 80 mM).

30 KDa) present only with RL5, 6 and 7 with Giza 843 under 10 mM NaCl and with RL 8 in all NaCl concentrations (Figure 2). Surprisingly the salt sensitive faba bean cultivar. Sakha1 showed the same unique bands (29 and 30 KDa) in most of the *Rhizobium* strains and NaCl concentrations. Our SDS protein profile support

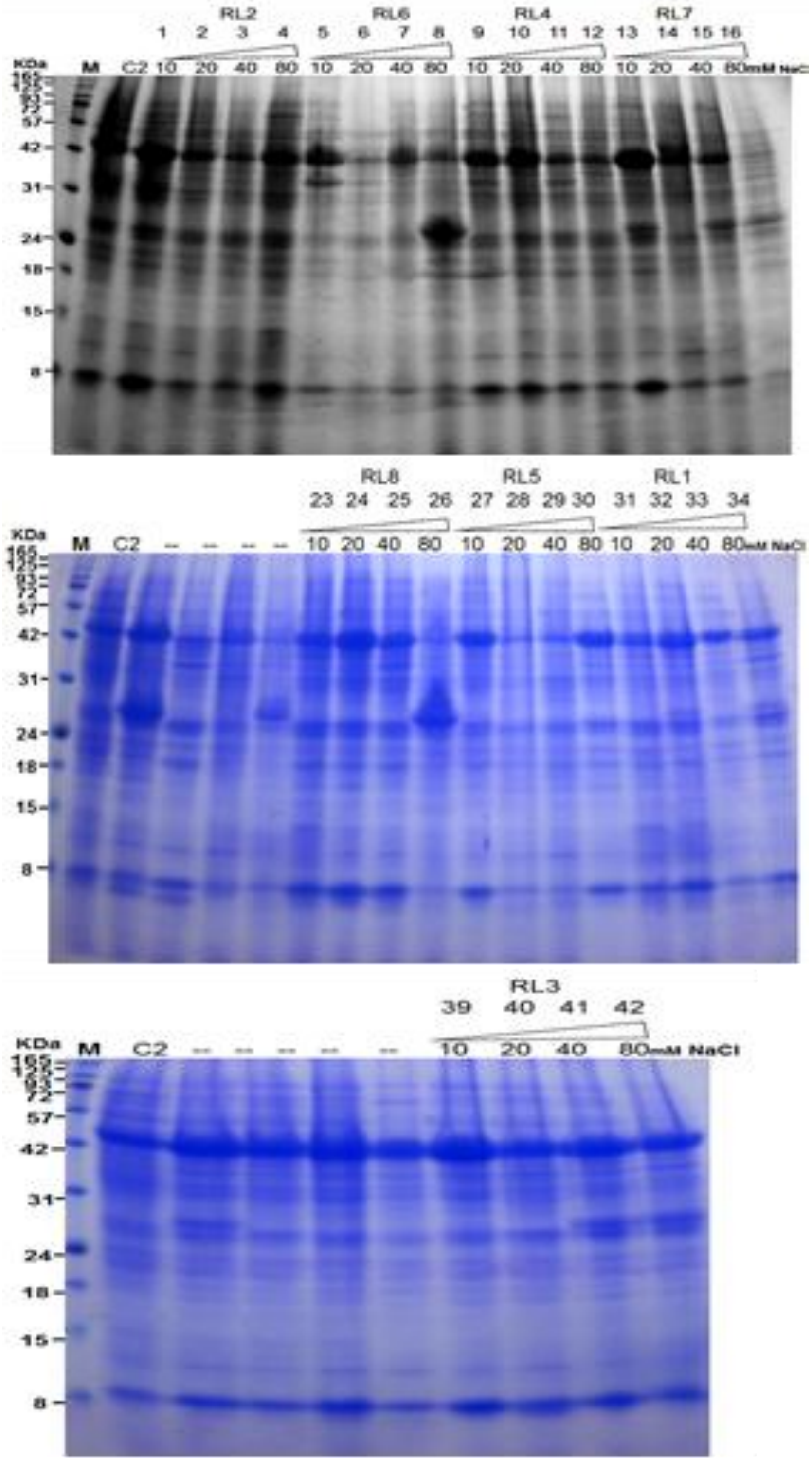

Figure 2. SDS-PAGE soluble protein profile of the interaction between faba bean Giza843 cultivar and the eight *Rhizobium* strains (RL 1, 2, 3, 4, 5, 6, 7 and 8) under four different concentration of NaCl (10, 20, 40 and 80 mM).

the positive effect of the *Rhizobium* strains on faba bean cultivars which showed high phosphor and nitrogen content like RL3, RL4 and RL8 in spite of their quantitative decrease of their fresh, dry weight, plant height and number of nodules measurements.

RL2 and RL3 interaction protein profile with both faba bean cultivars reveals a very stable and adapted behavior to the NaCl treatment. The decrease in soluble protein content of nodules is a general response to salt stress of many legume plants while, Fahmi, et al. (2011) reported that, the decrease in soluble protein of the nodules may be due to a protein breakdown or to an alteration in the incorporation of amino acids into protein. However, the importance of nutritional value in faba bean is based on its high protein concentration and symbiotically fixed nitrogen with Rhizobia (Mudgal et al., 1997). Few bands (29 and 30 KDa) were observed with the *Rhizobium* strains RL1, 3 and 8 in both faba bean cultivars under high concentrations of NaCl 40 and 80 mM. Zahran (1999) who reported the appearance of new protein bands in SDS-PAGE profiles of rhzobia from woody legumes grown under salt stress had reported similar results.

These two bands based on their molecular size are likely to be nitrogen fixation proteins. The nitrogen fixation regulatory proteins (NIFL) involved in regulation of transcriptional activation of nitrogen fixation and removal radicals contributed in environmental stress like falavoproteins (Heazlewood et al., 2004; Araujo et al., 2010). On the contrary, one approach to understanding the ability of *Rhizobium* to tolerate salt stress has been to identify stress-induced changes of individual proteins under the assumption that stress adaptation results from alterations in gene expression.

Conclusion

In the present study, the interactions between these strains of *R. leguminosarum* and faba bean varied based on several factors like plant genotype and *Rhizobium* strains. Nitrogen fixation response to salt varied between the eight *Rhizobium* strains. Four *Rhizobium* strains (RL1, 2, 4 and 8) showed tolerance under concentrations 40 and 80 mM of NaCl with both faba bean cultivars. The present results, it may be concluded that salt stress affects the growth, metabolic activity and symbiotic efficiency of different strains of *Rhizobium* and faba bean cultivars. However, important variability was observed amongst the sensitive and tolerant faba bean cultivars. An extensive work need to be done to identify these protein bands and analyzed their function in nitrogen fixation under salt stress in Egyptian environment conditions.

Conflict of Interests

The author(s) have not declared any conflict of interest.

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