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REVIEW ARTICLE

INFLUENCE OF *Rhizobium* ON THE GROWTH AND SYMBIOTIC PERFORMANCE OF *Arachis hypogaea* L. UNDER THE SALT STRESS CONDITION

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ABSTRACT

Rhizobium-legume symbiosis is one of the most well-established symbiotic nitrogen-fixing systems for agronomic studies. The effect of salt stress on the growth and nitrogen assimilation of *Arachis hypogea* (groundnut) was investigated. Root nodules were collected from healthy plants of *A.hypogaea* from the agricultural fields of Perambalur, Tamil Nadu, India. The Rhizobiaisolates obtained from pure cultures were subjected to standard biochemical tests. *A. hypogea* cultivated in pots were treated with saline water at 40mM, 80mM, 120mM and 160Mm. Plants not treated with saline water served as control. The plants inoculated with *Rhizobium*can grow and survive at high salt concentrations compared to control plants. In order to further understand the nitrogen fixing capability, the nitrogenous activity and leghaemoglobin content was determined. It was observed that the plants inoculated with *Rhizobium* showed increased nitrogenase activity and leghaemoglobin content. The overall conclusion is that appropriate legume and *Rhizobia* inoculants can increase the Nitrogen fixing capacity which further helps in improved food production even under stressed environmental conditions.

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INTRODUCTION

Biological Nitrogen Fixation (BNF) is an effective alternative natural source of nitrogen made available to the soil (Predeepa and Ravindran, 2010). In legumes, this process depends on the compatible interaction between the plant and soil bacteria referred to as rhizobia (Benjamin Gourion *et al.,* 2014). However, several environmental conditions are limiting the efficient growth and activity of nitrogen-fixing plants (Osuagwu *et al.,* 2014). Soil salinization is one of the largest constrains to plant productivity threatening agricultural land throughout the world (Osuagwu *et al.,* 2014; El-Akhal *et al.,* 2012). It affects the root nodule bacteria and their nitrogen fixing capacity. Legumes behave differently under saline conditions. Their responses is dependent on a number of factors which include the concentration of salt in the soil solution (Osuagwu *et al.,* 2014). As the most serious threat faced by agriculture in arid and semi-arid regions is salinity, selecting a salt tolerant rhizobial strain or a salt tolerant legume, the probability for the success of the partnership has been a failure. This is because legume-*Rhizobium* symbiosis and nodule formation on the legumes is more sensitive to salt or osmotic stress than the *Rhizobia* or the plant (Predeepa and Ravindran, 2010).

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The bacteria which form nitrogen fixing symbiosis with legume plants belongs to a diverse group of α and β proteobacteria and are called *Rhizobia* (Gergely Maroti and Eva Kondorosi, 2014). These bacteria are capable of inducing the development of and then populating nitrogen-fixing nodules (Robert Walker *et al.,* 2015). *Rhizobium* is the groups of small, rod shaped, Gram negative bacteria within which several strains nodulate a common host, but are distinct according to genetic and / or phenotypic properties (eg. *R.Tropici* and *R.etli*). However some strains cannot be distinguished other than by their host range, therefore the species is further classified into biovars (bv) (eg. *R. Leguminosarum* is split into three biovars that nodulate clover, peas and beans. *Rhizobium* has been isolated from legume plants such as alfalfa, soybean, groundnut etc, where symbiosis between legumes and rhizobia is a result of interactions among plants, bacteria and their environments (Meenu Saraf and DharaPatel, 2014).

It belongs to the family Fabacea (Leguminosae) and the Papillionacae sub-family. It is a perennial or annual legume with three or four leaflets, stipulated leaves, papillonate flowers and subterranean fruit. The plants are low sub-erect herbs often prostate and even creeping. The stem is generally angular, pubscent and solid with large central pith in early stage and stems tend to become hollow and cylindrical and shed most of their hair especially on the lower internodes. A.

hypogea has many uses; they can be eaten raw, used in recipes, made into solvents and oils, medicine, textile materials and peanut butter (Osuagwu *et al.,* 2014). The groundnut is a useful source of fat (35%-54%) and is very important in crop rotation system, as they help in Biological Nitrogen Fixation. Like many other leguminous crop species, peanut is relatively sensitive to drought and salinity. Salinity decreases peanut germination and seedling growth, dry matter production induces damage to the photosynthetic apparatus, Ca, K and Mg deficiencies and important yield losses. Saline conditions may severely limit peanut N fixation by reducing *Rhizobia* viability and also reducing nodule number, size and dry weight (El-Akhal *et al.,* 2012). The major N_2 fixing systems are the *Rhizobium*-legume symbiotic system which plays an important role in soil fertility. The *Rhizobium*-legume symbiosis is affected by various environmental conditions. The present investigation aims to contribute to the understanding of the *Rhizobium* -legume symbiotic system in order to increase the productivity and tolerance of *Arachis hypogaea* to the salt stress conditions.

MATERIALS AND METHODS

Sample collection: *Arachis hypogaea* plant was collected from the agricultural fields of Perambalur, Tamil Nadu, India.

Collection of nodules (Vincent, 1970)

The legume plants were uprooted and loosely adhering soil was removed by gentle shaking. The roots along with mature nodules were thoroughly washed in running water until the removal of adhering soil particles. The collected nodules were kept in sterile polythene bags and transported to the laboratory for further investigation.

Isolation and purification of *Rhizobium* from root nodules (Vincent, 1970)

The collected nodules were washed four tofive times with sterile distilled water, surface sterilized using 0.1% $Hgcl_2$ solution for 1 min, 70% C_2H_5OH for 4-5 min and washed in distilled water; it was then transferred to 70% C_2H_5OH for 2 min and finally washed in distilled water to remove all the traces of sterilants.

The sterilized root nodules were crushed in pestle and mortar by adding small aliquots of sterile water of 10^{-1} dilution. This suspension was serially diluted up to 10^{-7}. The diluted suspensions 10^{-5}-10^{-7} were inoculated with 0.1 ml of suspension in sterile petri-plates containing Yeast Extract Mannitol Agar medium [YEMA] with congo red. The inoculated plates were incubated at 30 ± 2°C for three days. At the end of the incubation period, the Rhizobial colonies appeared white, translucent and elevated. They were picked out using a sterile inoculating loop and uniformly streaked on YEMA medium. The Rhizobial isolates were purified, sub-cultured and stored for further investigation.

Identification of *Rhizobium* Species

Pure cultures of the isolates were made and then subjected to Gram reaction. The Gram negative isolates were further subjected to biochemical tests including catalase, oxidase, voges-Proskauer and indole tests for confirmation. Motility test was carried out to test for motility using flagella mordant (Loffler's mordant) (Agah *et al.,* 2016).

Pot experiments

According to Wahab *et al.* (2002) the pot experiments were carried out in the experimental form at P.G. and Research Department of Botany, Jamal Mohamed College, Trichy, Tamil Nadu. Experiments were carried out in pots filled with 2 kg soil, previously heat sterilized in metal buckets of 100°C for 1 hour on each of the three successive days. This is a recognized technique for soil sterilization since some spore forming bacteria may tolerate high temperatures, spores may germinate on the second or third day. However, soil and seeds were initially sterilized in order to eliminate possible contamination by resident Rhizobia, later plants were kept under non-sterile conditions. Pots were sterilized by swabbing thoroughly with 95% (v/v) C_2H_5OH.

Soil analysis (Chapman and Pratt, 1982)

Soil used in this study was collected from Perambalur Agricultural Land, Tamil Nadu. The soil used in this study was screened in 2mm sieve and the gravel content was discarded. The remainder was kept for mechanical and chemical analysis. Percentages of sand, silt and clay were calculated. For the chemical analysis, chlorides, bicarbonate calcium, magnesium, sodium and potassium were determined using the saturated soil paste extract method. The conductivity (EC) of the saturated soil paste extract was determined and the pH of a saturated soil paste was also estimated. Carl-zeiss flame photometer was used for sodium and potassium determinations. This same soil was used for all experiments.

Sterilization of seeds

Seeds of *Arachishypogeae* was surface sterilized by rinsing in C_2H_5OH (90% v/v) and soaking for 5 minutes in H_2O_2 (3% v/v) followed by three washings in sterile distilled water.

Germination of seeds

Seeds were germinated in sterilized dishes containing sterile damp filter paper .Sterile distilled water was added at intervals to keep the filter paper and germinating seeds wet. Seeds were incubated at 30°C for 2-3 days until radicals were 2-3 cm long and root hairs appeared. Three or four seedlings were inoculated with *Rhizobium* culture.

Preparation of inoculums

Rhizobium isolate from *Arachis hypogaea* plants were used for pot experiments. The bacteria were grown in 250 ml Erlenmeyer flasks containing 40 ml yeast-extract mannitol (YEM) broth (Somasegaran and Hoben, 1985) in a shaking incubator for 3 days. The cultures were shaken only for 8 hrs each day at 28°C. One mille liter (containing 10^{6-7} cells) of the bacterial culture at their logarithmic stage of growth was inoculated just after seedlings were transferred into the pots. Three days later, seedlings were re-inoculated in order to confirm root hair infection by *Rhizobia*.

Nutrient solution

Pots were surface irrigated once or twice weekly, according to the prevailing climatic conditions, alternatively with water and

a nutrient solution of the following composition (g l^{-1}): K_2HPO_4, 0.2; $(NH_4)_2 SO_4$, 0.03; $MgSO_4.H_2O$, 0.2; FeCl, 0.01; $CaCL_2$, 0.376; K_2SO_4, 0.845. The nutrient solution was almost free of N; only small amounts of NH_4NO_3 (0.1 g l^{-1}) were added to initiate the growth and nodulation of plants. Microelements were of the following composition (mg l^{-1}): H_3RO_4, 1.855; $MnSO_4.4H_2O$, 2.231; $ZnSO_4.7H_2O$, 0.288; $CuSO_3.5H_2O$, 0.25; Na_2MoO_4, 0.412. The pH of the solution was adjusted to 6.9 using KOH.

Salt stress treatment

After transplanting the sterile seedlings into pots, the inoculated plants were irrigated by the nutrient solution described above. Treatments were imposed when plants were 21 days old. Preliminary experiments showed that plants can form active N_2-fixing nodules at that age, accordingly, treatments in the present study started after module formation on plant roots. In addition to controls, four levels of salinity were applied where NaCl was added to the basic nutrient solution as follows:

S_1: 40 mMNaCl(4 dsm^{-1}). S_2: 80mM NaCl (9.3 dsm^{-1}).
S_3: 120 mMNaCl(11.0 dsm^{-1}) and 160 mMNaCl (13.9 dsm^{-1}).

As NaCl is known to be absorbed by several legumes (Youssef and Sprent, 1983), 50 ml of each level as applied once in every 10 days and salt treatments continued for 9 weeks. At the end of each period (10 days), the pots were flushed thoroughly with non-saline nutrient solution to avoid salt precipitation around roots. As plants grew in size with healthy nodules, the volume of liquid added was increased. The proportional difference between salt treatments was kept constant.

Determination of morphometric characters of plants, fresh and air-dry weight of nodules

Plants grown for the salt stress studies were harvested at 10, 25, 40 and 55 days after treatments. Each plant was decapitated and the root length, shoot length, leaves length and width were measured. The root systems were washed gently under tap water. Roots were blot dried and nodules from each individual root were collected, counted and air-dried. Fresh weight of nodules was estimated. Nodules were air-dried at room temperature for 4 h or until their weight was constant.

Estimation of nitrogenase activity by the acetylene reduction technique (Hardy *et al.*, 1968)

Nitrogenase activity was determined using a closed system on detached root system and the acetylene reduction assay was employed. For C_2H_2 reduction assays, undisturbed roots, cut off at cotyledonary nodes were placed in 250 ml mannitol bottles and sealed with a rubber septum and immediately injected with C_2H_2 to yield 10% final concentration. The bottles were then incubated at 28°C for 1 h and the reaction was terminated using 6 m HCl. A 0.5 ml gas sample was injected into a PyeUnicam FID 104 gas chromatograph fitted with a 4 ft coiled glass column packed with activated alumina at 150°C. The carrier gas was pure nitrogen at 40 ml min^{-1}. Two controls to check indigenous production of C_2H_4 were assayed.

Nodule fractionation

Preparation of nodule cytosol: Half the amount of the homogenate prepared in (A) was used for the cytosol fraction. The homogenate was filtered through four layers of cheesecloth and the filtrate was centrifuged at 3500 g for 10 min to remove nodule debris. The supernatant was centrifuged at 20,000 rpm (2-4°c) for 20 min and the resulting supernatant nodule cytosol was used for the determination of leghaemoglobin.

Determination of leghaemoglobin content (Becana*et al.*, 1986)

Leghaemoglobin determination was based on the absorption peak of cyanmethemoglobin under the influence of $C_6N_6FeK_3$ and KCN at 540 nM.

Statistical analysis

All values were means of 5 replicates per treatment. All the results were subjected to multifactorial analysis of variance subjected (ANOVA). Data are presented in terms of mean, standard deviation, standard error and coefficient of variation.

RESULTS AND DISCUSSION

In this present study, strains of root modulating bacteria were isolated from the root nodules of *Arachis hypogaea which* was collected from the agricultural fields of Perambalur, Tamil Nadu, India. All the rhizobial isolates showed dominant growth on YEMA medium and most were fast growers. Mostly growth of Rhizobia was obtained on 3 days of incubation; however some isolates were slow growers and were obtained after 3-5 days. Some studies already showed the fast-growing Rhizobia are more common in arid regions. This feature is a survival strategy, since they are more drought tolerant than slow-growing and multiply rapidly in a short period of wet weather, which would explain its greater frequency in soils of semiarid regions (Agah *et al.,* 21016). The *Rhizobium* colony morphology on YEMA medium was mostly circular, mucoid, white and translucent. The mucoid production would represent a mechanism involved in the process of adaptation and survival of *Rhizobium* in adverse conditions of soil and climate (Agah *et al.,* 21016). The fast growers failed to absorb congo red in the medium; Pseudo-nodule forming bacteria *Agrobacterium* utilized congo red but *Rhizobium* strains did not utilize congo red. This test is essential to differentiate *Rhizobium* and *Agrobacterium* (Vishal Kumar Deshwal and Abhishek Chaubey, 2014). Under the light microscope all the isolates were non-motile, gram negative and rod shaped bacteria. The biochemical tests performed on the isolates showed that they were positive to Indole, Nitrate Reduction, Urease, Catalase, Oxidase and Voges-Proskuer tests. These findings are in close agreement with Agah et al (2016) who have previously characterized the Rhizobium from soil and root nodules of groundnut with same positive biochemical tests.

Nodulation and morphological variations of *Arachis hypogaea* plants under salt stress

The data presented in tables 1 and 8 showed Arachis hypogaea plants grown on the different salinity levels. Root length, shoot length, nodule numbers, nodule fresh weight and nodule dry weights were gradually, but significantly reduced with the increase of NaCl level. *Rhizobium* inoculated plants showed increased plant growth characters and nodulation over uninoculated plants.

Table 1. Effect of different levels of NaCl on nodulation and morphometric characters of control plants of *Arachis hypogaea*– 10 DAT

Treatment	Root length (cm)	Shoot length (cm)	Leaves length (cm)	Leaves width (cm)	Nodule (number/plant)	Nodule fresh weight (g)	Nodule dry weight (g)
S_0	10^{a}	14.2^{a}	4.1^{a}	2.1^{a}	42^{a}	0.33^{a}	0.13^{a}
S_1	9^{a}	13^{a}	3.5^{ab}	1.7^{ab}	56^{c}	0.32^{a}	0.12^{a}
S_2	10^{a}	15.5^{a}	4^{a}	2^{a}	38^{a}	0.28^{a}	0.10^{ab}
S_3	13^{b}	16^{a}	4^{a}	2^{a}	34^{a}	0.31^{a}	0.12^{a}
S_4	8^{ab}	11^{b}	3.5^{ab}	1.8^{a}	28^{ab}	0.26^{ab}	0.09^{c}
SEd	0.49	0.69	0.18	0.09	4.26	0.03	0.01
CV%	17.35	17.44	16.84	16.76	17.93	16.87	17.04
SE	1.73	2.43	0.64	0.32	7.10	0.05	0.019
CD at 5% level	1.04	1.46	0.39	0.19	4.26	0.03	0.01
	S	S	NS	NS	S	NS	S

S_0 – Control (no addition of NaCl), S_1 – 40 mMNaCl (4 dsm^{-1}), S_2 – 80 mMNaCl (9.3 dsm^{-1}), S_3 – 120 mM (11 dsm^{-1}),
S_4 – 160 mM(13.9 dsm^{-1}), SEd – Standard deviation, CD – Critical difference at 5% probability level,
CV% - Coefficient of variation, SE – Standard error, S – Significant, NS – Not significant.
Values superscript with different letters on the same row indicates significant differences.

Table 2. Effect of different levels of NaCl on nodulation and some morphometric characters of *Arachis hypogaea* inoculated with *Rhizobium* – 10 DAT

Treatment	Root length (cm)	Shoot length (cm)	Leaves length (cm)	Leaves width (cm)	Nodule (number/plant)	Nodule fresh weight (g)	Nodule dry weight (g)
S_0	15.8^{a}	18.3^{a}	4a	2.1^{a}	65^{a}	0.29^{a}	0.17^{a}
S_1	10^{c}	15^{b}	3.5^{b}	1.7^{b}	52^{b}	0.24^{b}	0.16^{a}
S_2	16^{a}	17^{a}	4.1^{a}	2^{a}	60^{a}	0.28^{a}	0.17^{a}
S_3	12^{b}	16^{a}	4.2^{a}	2.1^{a}	45^{c}	0.21^{c}	0.14^{b}
S_4	9.5^{c}	10^{c}	3.5^{b}	1.5^{c}	30^{cd}	0.19^{d}	0.11^{c}
SEd	0.64	0.77	0.18	0.09	2.61	0.01	0.01
CV%	17.92	17.85	16.92	17.28	18.28	17.53	17.66
SE	2.26	2.72	0.65	0.32	9.21	0.04	0.02
CD at 5% level	1.36	1.63	0.39	0.19	5.52	0.03	0.02
	S	S	NS	S	S	S	S

S_0 – Control (no addition of NaCl), S_1 – 40 mMNaCl (4 dsm^{-1}), S_2 – 80 mMNaCl (9.3 dsm^{-1}), S_3 – 120 mM (11 dsm^{-1}),
S_4 – 160 mM(13.9 dsm^{-1}), SEd – Standard deviation, CD – Critical difference at 5% probability level,
CV% - Coefficient of variation, SE – Standard error, S – Significant, NS – Not significant.
Values superscript with different letters on the same row indicates significant differences.

Table 3. Effect of different levels of NaCl on nodulation and some morphometric characters of control plants of *Arachis hypogaea*– 25 DAT

Treatment	Root length (cm)	Shoot length (cm)	Leaves length (cm)	Leaves width (cm)	Nodule (number/plant)	Nodule fresh weight (g)	Nodule dry weight (g)
S_0	19.2^{a}	23.4^{a}	4.1^{a}	2.1^{a}	120^{a}	0.42^{a}	0.21^{a}
S_1	12^{c}	19^{b}	3.6^{ab}	1.8^{bc}	90^{b}	0.36^{b}	0.18^{b}
S_2	17^{ab}	22^{a}	4^{a}	2.5^{a}	120^{a}	0.41^{a}	0.21^{a}
S_3	18^{a}	23^{a}	4^{a}	2.2^{a}	122^{a}	0.45^{a}	0.19^{b}
S_4	13^{c}	18^{b}	3.5^{ab}	1.8^{a}	75^{c}	0.25^{c}	0.14^{c}
SEd	0.77	1.02	0.18	0.10	5.29	0.02	0.01
CV%	17.25	17.05	16.85	17.52	17.75	17.84	17.54
SE	2.73	3.59	0.64	0.36	18.71	0.06	0.03
CD at 5% level	1.64	2.16	0.39	0.22	11.22	0.04	0.02
	S	NS	NS	S	S	S	S

S_0 – Control (no addition of NaCl), S_1 – 40 mMNaCl (4 dsm^{-1}), S_2 – 80 mMNaCl (9.3 dsm^{-1}), S_3 – 120 mM (11 dsm^{-1}),
S_4 – 160 mM(13.9 dsm^{-1}), SEd – Standard deviation, CD – Critical difference at 5% probability level,
CV% - Coefficient of variation, SE – Standard error, S – Significant, NS – Not significant.
Values superscript with different letters on the same row indicates significant differences.

Table 4. Effect of different levels of NaCl on nodulation and some morphometric characters of *Arachis hypogaea* inoculation with *Rhizobium* – 25 DAT

Treatment	Root length (cm)	Shoot length (cm)	Leaves length (cm)	Leaves width (cm)	Nodule (number/plant)	Nodule fresh weight (g)	Nodule dry weight (g)
S_0	28.2^{a}	22^{a}	4.2^{a}	2.1^{a}	212^{a}	0.50^{a}	0.25^{a}
S_1	20.6^{c}	15^{c}	3.5^{b}	1.7^{b}	150^{bc}	0.49^{a}	0.24^{a}
S_2	30^{a}	20.5^{a}	4.2^{a}	2.1^{a}	205^{a}	0.48^{a}	0.25^{a}
S_3	30^{a}	21^{a}	4.3^{a}	2.2^{a}	185^{a}	0.44^{ab}	0.22^{ab}
S_4	26^{b}	16^{bc}	3^{c}	1.5^{c}	129^{c}	0.32^{c}	0.16^{c}
SEd	1.28	0.92	0.19	0.09	8.82	0.02	0.01
CV%	16.88	17.18	17.40	17.41	17.70	17.61	17.69
SE	4.53	3.24	0.66	0.33	31.19	0.07	0.039
CD at 5% level	2.72	1.95	0.40	0.20	18.70	0.05	0.02
	S	S	S	S	S	S	S

S_0 – Control (no addition of NaCl), S_1 – 40 mMNaCl (4 dsm^{-1}), S_2 – 80 mMNaCl (9.3 dsm^{-1}), S_3 – 120 mM (11 dsm^{-1}),
S_4 – 160 mM(13.9 dsm^{-1}), SEd – Standard deviation, CD – Critical difference at 5% probability level,
CV% - Coefficient of variation, SE – Standard error, S – Significant, NS – Not significant.
Values superscript with different letters on the same row indicates significant differences.

Table 5. Effect of different levels of NaCl on nodulation and some morphometric characters of control plants of *Arachis hypogaea*– 40 DAT

Treatment	Root length (cm)	Shoot length (cm)	Leaves length (cm)	Leaves width (cm)	Nodule (number/plant)	Nodule fresh weight (g)	Nodule dry weight (g)
S_0	24^{a}	22.8^{a}	4.5^{a}	2.3^{a}	232^{a}	0.68^{a}	0.34^{a}
S_1	15^{b}	20.5^{a}	3.7^{b}	1.9^{b}	200^{ab}	0.6^{ab}	0.3^{ab}
S_2	21^{ab}	22.5^{a}	4.4^{a}	2.2^{a}	224^{a}	0.66^{a}	0.32^{a}
S_3	22^{a}	21^{a}	4.2^{a}	2.1^{a}	150^{b}	0.54^{b}	0.26^{b}
S_4	17^{b}	18^{ab}	3.5^{b}	1.6^{b}	97^{c}	0.44^{c}	0.2^{c}
SEd	0.96	1.01	0.20	0.10	9.54	0.03	0.01
CV%	17.13	17.08	17.08	17.32	18.66	17.57	17.74
SE	3.39	3.58	0.69	0.35	33.71	0.10	0.05
CD at 5% level	2.03	2.15	0.42	0.21	20.22	0.06	0.03
	S	S	S	S	S	S	S

S_0 – Control (no addition of NaCl), S_1 – 40 mMNaCl (4 dsm^{-1}), S_2 – 80 mMNaCl (9.3 dsm^{-1}), S_3 – 120 mM (11 dsm^{-1}),
S_4 – 160 mM(13.9 dsm^{-1}), SEd – Standard deviation, CD – Critical difference at 5% probability level,
CV% - Coefficient of variation, SE – Standard error, S – Significant, NS – Not significant.
Values superscript with different letters on the same row indicates significant differences.

Table 6. Effect of different levels of NaCl on nodulation and some morphometric characters of *Arachis hypogaea* inoculated with *Rhizobium* – 40 DAT

Treatment	Root length (cm)	Shoot length (cm)	Leaves length (cm)	Leaves width (cm)	Nodule (number/plant)	Nodule fresh weight (g)	Nodule dry weight (g)
S_0	32.4^{a}	25.8^{a}	4.4^{a}	2.2^{a}	375^{a}	1.3^{a}	0.67^{a}
S_1	25.3^{b}	18^{b}	3.2^{b}	1.6^{b}	310^{b}	0.84^{b}	0.43^{b}
S_2	29.6^{a}	24.5^{a}	4.3^{a}	2.2^{a}	360^{a}	1.20^{a}	0.64^{a}
S_3	30^{a}	23^{ab}	4.4^{a}	2.3^{a}	220^{bc}	0.76^{bc}	0.41^{b}
S_4	18.8^{c}	17^{bc}	3.2^{b}	1.8^{b}	150^{cd}	0.6^{cd}	0.3^{c}
SEd	1.36	1.07	0.19	0.10	15.06	0.05	0.03
CV%	17.69	17.46	17.30	17.06	18.81	18.43	18.57
SE	4.81	3.78	0.67	0.34	53.25	0.17	0.09
CD at 5% level	2.89	2.27	0.40	0.21	31.94	0.10	0.05
	S	S	S	S	S	S	S

S_0 – Control (no addition of NaCl), S_1 – 40 mMNaCl (4 dsm^{-1}), S_2 – 80 mMNaCl (9.3 dsm^{-1}), S_3 – 120 mM (11 dsm^{-1}),
S_4 – 160 mM(13.9 dsm^{-1}), SEd – Standard deviation, CD – Critical difference at 5% probability level,
CV% - Coefficient of variation, SE – Standard error, S – Significant, NS – Not significant.
Values superscript with different letters on the same row indicates significant differences.

Table 7. Effect of different levels of NaCl on nodulation and some morphometric characters of control plants of *Arachis hypogaea*– 55 DAT

Treatment	Root length (cm)	Shoot length (cm)	Leaves length (cm)	Leaves width (cm)	Nodule (number/plant)	Nodule fresh weight (g)	Nodule dry weight (g)
S_0	25.8^{a}	23.5^{a}	4.5^{a}	2.3^{a}	250^{a}	0.9^{a}	0.42^{a}
S_1	18^{b}	17^{b}	4^{ab}	2.3^{a}	210^{b}	0.13^{b}	0.36^{b}
S_2	24.4^{a}	22.8^{a}	4.5^{a}	2.2^{a}	235^{a}	0.82^{a}	0.4^{a}
S_3	22.2^{a}	21.4^{a}	4.4^{a}	2.1^{a}	162^{bc}	0.7^{b}	0.35^{b}
S_4	17.6^{b}	16^{b}	3.7^{ab}	2^{a}	105^{c}	0.54^{c}	0.27^{c}
SEd	1.06	0.99	0.20	0.10	10.11	0.04	0.02
CV%	17.35	17.42	17.01	16.83	18.58	17.59	17.52
SE	3.74	3.5	0.71	0.36	35.75	0.12	0.06
CD at 5% level	2.25	2.10	0.43	0.22	21.44	0.08	0.04
	S	NS	NS	S	S	S	S

S_0 – Control (no addition of NaCl), S_1 – 40 mMNaCl (4 dsm^{-1}), S_2 – 80 mMNaCl (9.3 dsm^{-1}), S_3 – 120 mM (11 dsm^{-1}),
S_4 – 160 mM(13.9 dsm^{-1}), SEd – Standard deviation, CD – Critical difference at 5% probability level,
CV% - Coefficient of variation, SE – Standard error, S – Significant, NS – Not significant.
Values superscript with different letters on the same row indicates significant differences.

Table 8. Effect of different levels of NaCl on nodulation and some morphometric characters of *Arachis hypogaea* inoculated with *Rhizobium* – 55 DAT

Treatment	Root length (cm)	Shoot length (cm)	Leaves length (cm)	Leaves width (cm)	Nodule (number/plant)	Nodule fresh weight (g)	Nodule dry weight (g)
S_0	29.5^{a}	27.4^{a}	4.6^{a}	2.3^{a}	405^{a}	1.4^{a}	0.75^{a}
S_1	21.4^{b}	21^{b}	3.8^{ab}	1.9^{ab}	310^{b}	0.94^{b}	0.48^{c}
S_2	26.7^{a}	27.5^{a}	5^{a}	2.5^{a}	370^{a}	1.3^{a}	0.7^{a}
S_3	27^{a}	27.2^{a}	5^{a}	2.5^{a}	317^{b}	1.25^{a}	0.6^{a}
S_4	19^{c}	19^{bc}	4^{ab}	2^{ab}	168^{c}	0.87^{b}	0.46^{c}
SEd	1.22	1.21	0.22	0.11	16.38	0.06	0.03
CV%	17.41	17.47	17.09	17.09	18.43	17.57	17.62
SE	4.30	4.26	0.76	0.38	57.89	0.20	0.10
CD at 5% level	2.58	2.56	0.46	0.23	34.72	0.12	0.06
	S	S	NS	NS	S	S	S

S_0 – Control (no addition of NaCl), S_1 – 40 mMNaCl (4 dsm^{-1}), S_2 – 80 mMNaCl (9.3 dsm^{-1}), S_3 – 120 mM (11 dsm^{-1}),
S_4 – 160 mM(13.9 dsm^{-1}), SEd – Standard deviation, CD – Critical difference at 5% probability level,
CV% - Coefficient of variation, SE – Standard error, S – Significant, NS – Not significant.
Values superscript with different letters on the same row indicates significant differences.

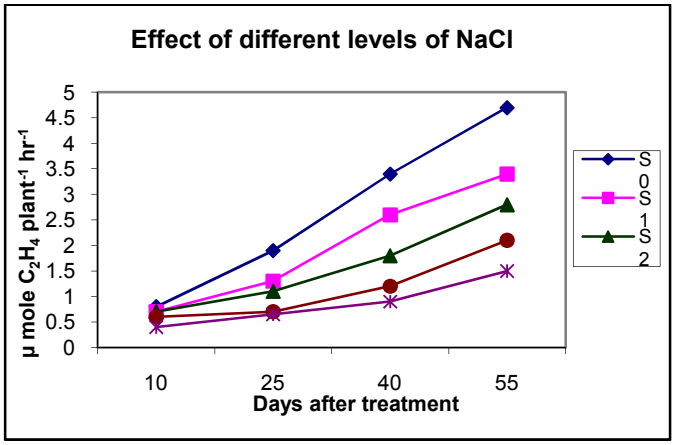

Figure 1. Nitrogenase activity in control plants of *Arachis hypogaea*

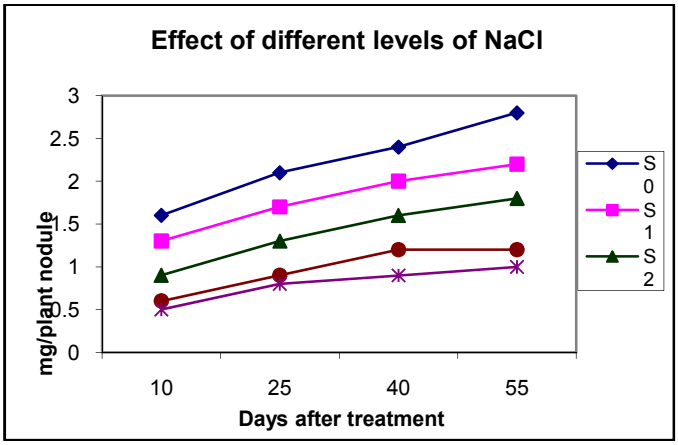

Figure 3. Leghaemoglobin content in control plants of *Arachis hypogaea*

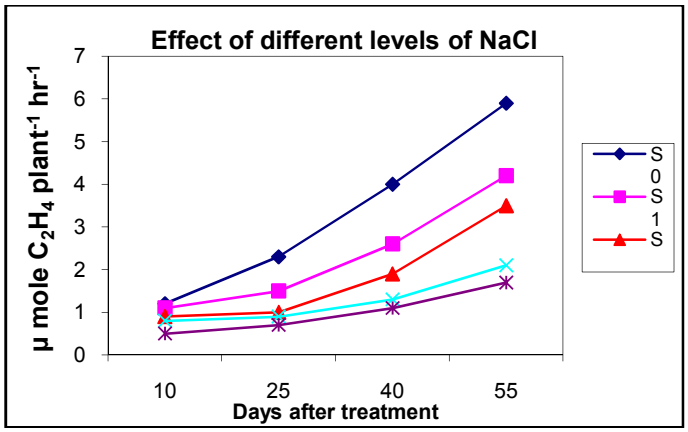

Figure 2. Nitrogenase activity in *Rhizobium* inoculated plants of *Arachis hypogaea*

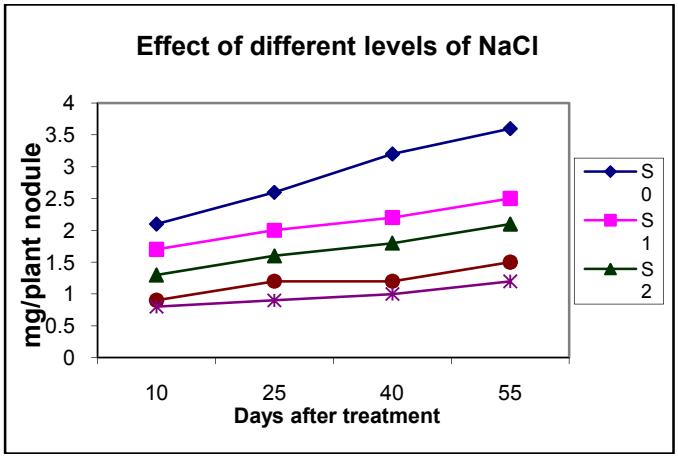

Figure 4. Leghaemoglobin content in *Rhizobium* inoculated plants of *Arachis hypogaea*

But plant leaves had no variation at different salinity levels. At and after 25 DAT root and shoot length, leaves area, nodule number, nodule fresh weight and nodule dry weights were higher in the *Rhizobium* inoculated plants when compared to control plants and also declined progressively with increased salinity levels. Low and high salt levels reduced the nodule number to about 40 per cent of non-salt treatment. Salt had more effect on the root and shoot length, however, nodule number and it weights were significantly reduced for all treatments but no significant different was observed in leaves. Roots appeared to be more sensitive to salinity than shoots of both plants. At 160 mMNaCl (S_4) both plants showed the significant reduction of the shoot and root length and nodulation in inoculated and uninoculated plants. These observations tend to agree with the reports of Osuagwu and Udogu (2014) Maggio *et al.,* (2007) and Neocleous and Vasilakakis (2007). The reduction in vegetative growth of *Arachis hypogaea* due to the effect of salt stress might be due to the effect of salinity on important cellular and metabolic processes in plants which include cell division, cell expansion, photosynthesis, protein synthesis, lipid metabolism, which might be due to the inability of the plants to take up water causing imbalance in osmotic potential, ionic equilibrium and nutrient up take. Salt stress is recorded to cause decreased biosynthesis of chlorophyll and inefficiency of photosynthesis which ultimately lead to lowered productivity. Decline in photosynthesis due to salinity might be due to lowered stomatal conductance, depression in carbon up take and metabolism, inhibition of photochemical capacity or the combination of all these factors (Osuagwu and Udogu (2014)).

Estimation of nitrogenase activity

Absolute nitrogenase (ARA/plant) activity of *Arachis hypogaea* of control and salt stressed plants is presented in figures: 1 and 2.Nitrogenase activity was significantly affected by salt stress.C_2H_2 reduction activity increased steadily with plant growth. Thus, 55 DAT of both varieties at their flowering stage and exhibited more N_2 fixing activity which was proportional to the nodule population. Plants treated with 80mM NaCl recorded about 70% activity of non-saline controls. S4 inoculated plants (160mM NaCl) recorded 33% ARA of the controls which are still considered as functioning nodules at this high levels of salinity. Butcontrol stressed plants ARA activity was decreased than inoculated plants. The measurement of nitrogenase activity was based on the reduction of acetylene to ethylene as quantities by gas chromatography. Nitrogenase activity of both plant nodules inoculated with rhizobial isolates showed maximum acetylene reduction than uninoculated stressed plants. The present study clearly showed that the leguminous plants, if free from *Rhizobium*, nodules and nitrogenase activity was decreased and also it values tend to increase significantly by increasing plant growth.

Determination of Leghaemoglobin content

Results in figures: 3 and 4 the leghaemoglobin content recorded in *Arachis hypogaea*. The values decreased at mid-salinity levels (40 and 80 mMNacl) and also decreased by salt

increments. But the LHB content was to increase with plant growth (55 DAT). The present study can be related to Hamdi Hussein Zahran (Hamdi Hussein Zahran, 1999) who worked soy bean and chick pea inoculated with strains of *Rhizobium leguminosarum*. The effect of salt stress on N_2 fixation by legumes is directly related to the salt-induced decline in dry weight and N content in the shoot. The salt-induced distortions in nodule structure could also be reasons for the decline in the N_2 fixation rate by legumes subject to salt stress. Reduction in photosynthetic activity might also affect N_2 fixation by legumes under salt stress (Hamdi Hussein Zahran, 1999).

Conclusion

The present study concludes that rhizobial isolates helped in increasing the growth and nodulation of *Arachis hypogaea* under salt stress condition. The symbiosis between Rhizobium and legume is a classic example of mutualism and are a cheaper and effective agronomic practice for supplying adqueate amount of nitrogen to the legume based crop and pasture. The rhizobium legume symbiosis can be used as an ideal solution for the improvement of soil fertility and rehabilitation of arid lands and hence is important for future result.

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