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Influence of *rhizobium* on the Growth and Symbiotic Performance of *Arachis hypogaea* L under the Water Stress Condition

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Abstract

Groundnut (*Arachis hypogaea* L) is a leguminous plant which is symbiosis with nitrogen fixing bacteria such as Rhizobium. During drought condition such as water stress, the plant is affected both chemically and physiologically. In this present study the Rhizobium was isolated and purified from *Arachis hypogaea* L in natural conditions and inoculum were stored for further study. The *Arachis hypogaea* L seeds were cultivated in lab conditions as both control and inoculated with Rhizobium. Both inoculated and control seeds were cultivated for several days by inducing water stress. After the investigated days the morphometric characters, nitrogenase activity and Leghaemoglobin content of both control and inoculated plants were determined in which the inoculated plants showed resistance towards the water stress and yield is high when compared to control plants during water stress.

1. Introduction

Rhizobium is the gram-negative, rod shaped small bacteria, which infects leguminous plants by producing nodules in roots. The successful *Rhizobium*- legume symbiosis increases the incorporation of BNF* into soil ecosystem. The atmospheric nitrogen fixed symbiotically by *Rhizobium*- legumes represents a renewable source of nitrogen for agriculture. Several environmental conditions are the limiting factors to the growth and activity of the nitrogen fixing plants and *Rhizobium.* There has been extensive study of the *Rhizobium* legume symbiosis, identifying many of the *Rhizobia* genes required for the nodulation and nitrogen fixation. Groundnut (*Arachis hypogaea* L) is an important oil seeded legume crop which contains 44–56% oil and 22–30% protein on a dry seed basis [1]. However, when fixing nitrogen, low level of oxygen are required to protect the enzyme nitrogenase and hence, rhizobium are able to grow in microaerophilic condition. These bacteria play a duel role i.e., free living forms soils and a symbiotic form inside of host legumes. In South Africa groundnut yield is reduced by 25.8% and in East Asia by 18.5% when compared to 1980s with 1970s due to drought conditions [2], [3]. In India groundnut yield is fluctuated from 550-1100 ha^{-1} in different years which leads to fall of total yield production by 4.3-9.6 million tons [4]. Drought stress affects the water relations [5], minerals, nutrition, photosynthesis [6], metabolism, growth, and yield of groundnut [7]. Drought condition also influence on growth of weeds, agronomic management, and also on intensity of insects, pests, and diseases [8]. Drought is

generally due to low relative humidity, high temperature and wind speed which in turn influence groundnut. The native rhizobial strains tolerated a higher salt concentration (5% NaCl) that the exotic rhizobial strains. Both native and exotic strains fails to grow at pH 4 and 4.5 levels in the laboratory conditions. In the soil adjusted to pH 4-7, all the native rhizobial strains persisted while those of the exotic strains failed to survive at pH below 5.5. The native stains were more versatile than the exotic ones in utilizing different carbohydrates as a sole carbon source and were found to be more resistant to many antibiotics (Streptomycine, chloramphenicol, rimfampenicillin, oxytetracycline, penicillin and tetracycline) that exotic strains which are found resistant to chloramphenicol only percentage of nitrogen fixation is also higher for native rhizobial strains these isolates being found to be superior to the exotic strains in stimulating growth, dry matter yield, nodulation and wet weight of faba bean in pouch culture.

Several environmental factors adversely affect plant growth and development and final yield performance of a crop [9]. Drought, salinity, nutrients in balances (including mineral toxicities and deficiencies) and extremes of temperature are among the major environmental constraints to crop productivity worldwide. Development of crop plants with stress tolerance, however, requires, among others, knowledge of the physiological mechanisms and genetic controls of the contribution traits at difference plant development stages. In the past 2 decades, biotechnology research has provided considerable insights into the mechanism of biotic stress tolerance in plants at the molecular level. Furthermore different abiotic stress factors may provoke osmotic stress, oxidative stress and protein denaturation in plants, which lead to similar cellular adaptive responses such as accumulation of compatible solutes, induction of stress protein and acceleration if reactive oxygen species scavenging systems. To improved plant tolerance to salinity injury through either chemical treatments or biofertilizers treatments (Asymbiotic nitrogen-fixing bacteria, symbiotic nitrogen-fixing bacteria and mycorrhiza) or enhance a process used naturally by plants to minimize the movements of Na^+ to the shoot, using genetic modification to amplify the process.

The response of the plants was analysed for three distinct stages of dehydration. In stage one, the rate of transpiration remained constant and equal to that of well watered plants even though soil water status fell by more than 50%. Stage two began when the rate of soil water supply to the plant was less than potential transpiration and stomata closed resulting in the maintenance of plant water balance. Stage three occurred once stomata had reached minimum conductance and water loss was then a function of the epidermal conductance and the environment around the leaf.

The present investigation aims to contribute the understanding of Rhizobium- *Arachis hypogaea* L. symbiotic relationship and increase the productivity and tolerance to the water stress.

2. Materials and Methods

2.1. Sample Collection

Groundnut plants were collected from agricultural field at Perambalur, Tamil Nadu. Healthy groundnut plants were uprooted and those plants possessing healthy nodules with pink colour were selected and transported to the laboratory in sterile polythene bag, for further investigation

2.2. Isolation and Purification of Rhizobium from Root Nodules

The collected nodules were washed with sterile distilled water surface sterilized using 0.1% mercuric chloride solution and finally washed with 70% ethanol and distilled water respectively to remove all traces of sterilents.

The sterilized root nodules were crushed with the help of pestle and mortar by adding small aliquots of sterile water, which was $10^{-1}.10^{-5}$ -10^{-7} were selected and 0.1ml of suspension was inoculated plates were incubated at 30± 2˚c for three days.

The *rhizobial* isolates were purified, subculture and stored for further investigation.

2.3. Identification of Rhizobium

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, methyl red, citrate utilization, hydrogen sulfide production, nitrate reduction, urease test, starch hydrolysis, gelatin hydrolysis, triple sugar iron agar, Mac-Conkey agar and indole tests for confirmation.

2.4. Pot Experiments

According to [10] the pot experiments were carried out in Research Department of Botany, Jamal Mohamed College, Trichy, Tamil Nadu. Experiments were carried out in pots filled with 2 kg soil were previously heat sterilized in metal buckets of 100 C for 1 hour on each of the three successive days.

2.4.1. Soil Analysis [11]

Soil used in this study was collected from Perambalur Agricultural Land, Tamil Nadu, India. The soil used in this study was screened in 2 mm sieve and the gravel content was discarded. The remainder was kept for mechanical and chemical analysis. Percentage of soil, slit and clay were calculated. For the chemical analysis, chlorides, bicarbonate calcium, magnesium, sodium and potassium were determined using the saturated soil paste extract method. Carl-zeiss flame photometer was used for all experiments.

2.4.2. Sterilization and Germination of Seeds

Seeds of *Arachis hypogeae* plants was surface sterilized by rinsing in ethanol (90% v/v) and soaking for 5minutes in

hydrogen peroxide (3% v/v) followed by three washings in sterile distilled water.

Seeds were germinated in sterilized dishes containing sterile damp filter paper and sterile distilled water and added at intervals to keep the filter paper germinating seeds wet. Seeds were incubated at 30 c for 2-3 days until radicals were 2-3 cm long and root hairs appeared.

2.4.3. Preparation of Inoculums and Nutrient Solution

Rhizobium isolates ARS 2 for *Arachis hypogaea* L plants were used for pot experiments and nodulation ability test. The bacteria were grown in 250 ml Erlenmeyer flasks containing 40 ml yeast-extract mannitol (YEM) broth in a shaking incubator for 3 days. After three days seedlings were re-inoculated in order to confirm root hair infection by rhizobia.

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; $MgSO_4.H_2O$, FeCl, 0.01; $CaCl_2$, 0.376; K_2SO_4, 0.845.

2.4.4. Water-Stress Treatments

The field capacity of the soils used was determined according to [11] and the water-potential levels from soils of similar texture, were found as follows.

(T_0 control, f.c.): (-0.16MPa), T_1:2/3 f.c. (-0.24 MPa), T_2:1/2 f.c (-0.32 MPa), T_3:1/3 f.c. (-0.49 MPa), T_4:1/6 f.c (-0.97 MPa).

The levels of water content were maintained constant for each treatment. Pots were weighed every other day and the water lost through evaporation and transpiration was replaced by adding water or nutrient solution equivalent to the loss by these factors.

2.5. Determination of Morphometric Characters of Plants and Fresh and Air-Dry Weight of Nodules

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

2.6. Estimation of Nitrogenase Activity by the Acetylene Reduction Technique [12]

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 Pye Unicam FID 104 gas chromatograph fitted with a 4 feet 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 ethylene were assayed.

2.7. Nodule Fractionation

2.7.1. 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 cheese cloth and the filtrate was centrifuged at 3500 g for 10 min to remove nodule debris. The supernatant was centrifuged at 20,000 g (2-4°C) for 20 min and the resulting supernatant nodule cytosol was used for the determination of leghaemoglobin.

2.7.2. Determination of Leghaemoglobin Content [13]

Leghemoglobin determination were based on the absorption peak of cyannmethaemoglobin under the influence of potassium ferricyanide and potassium cyanide at 540 NM.

2.7.3. Statistical Analysis

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

3. Results

3.1. Properties of Soil

The soil possess all essential micro and macro nutrients. Heavy metals were not found in the soil.

3.2. Nodulation and Morphological Changes of *Arachis hypogaea* L Plants under Water Stress

At 10 DAT*, the root and shoot length was significantly affected. Morphometric parameters of control plants (control) were shorter than inoculated ones. Result of the effect of different water regimes on *Arachis hypogaea* L plants growth are given in Table 1 and 2. Nodule numbers in *Arachis hypogaea* L plants inoculated with *Rhizobium* were 35, 30 and 22 at T_2, T_3 and T_4 treatments respectively, whereas 20, 18 and 12 (T_2, T_3 and T_4) was observed in control plants.

The data presented in Table 3 and 4 showed that water stress effect on growth of *Arachis hypogaea* L plant on 25 days after treatment. Plants were inoculated with *Rhizobium* strain performed better than control stressed plants.

Data presented in Table 5 and 6 showed that plant growth characters and nodulation was significantly reduced by water stress on 40 days after treatment. Under T_1 and T_4 level water conditions, the roots of control plants were shorter than inoculated ones. The nodule number and weight of stressed

control plants were reduced than the inoculated plants. Significant interactions was detected between water-conditions in inoculated stressed plants than the control stressed plants. At T_1 and T_2 level of water conditions (-0.24 and -0.32 MPa) the *Arachis hypogaea* L root length was increased from 23 to 46.5 cm (T_1) and 46 cm (T_2).

Drought stress greatly reduced the nodule number, data presented in Table 7 and 8 showed that plants harvested 55 days after treatment. The results of *Arachis hypogaea* L plant height showed that *Rhizobium* inoculation significantly enhanced root length (from 23 to 46.5 cm) and shoot length (from 23.2 – 34 cm).

Nodulation of *Arachis hypogaea* L and plants were significantly reduced by a decrease in soil moisture. However, the presence of more than 50% active nodules indicates that *Rhizobium* inoculated plants is tolerant to mild water defects, despite the decreasing values of nodule fresh weight with loss of available water. Nodules of inoculated plants yielded the greatest weights compared to control plants. Root and shoot length, nodule number, nodule fresh and dry weights declined only to 85 per cent of control (T_0 level) when subjected to T_2 and T_3 soil moisture, whereas T_4 treatment with the least soil water content yielded about 50 per cent of control.

Table 1. *Effect of different water regimes on nodulation and morphometric characters of control plants of Arachis hypogaea L – 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)
T_0	14.5^{a}	18^{a}	4.1^{a}	2.1^{a}	22^{a}	0.27^{a}	0.13^{a}
T_1	11^{b}	14.5^{b}	3.5^{ab}	1.7^{ab}	18^{b}	0.26^{a}	0.11^{ab}
T_2	13.5^{ab}	17^{a}	4^{a}	2^{a}	20^{a}	0.26^{a}	0.13^{a}
T_3	14^{a}	16^{a}	4^{a}	2^{a}	18^{b}	0.18^{b}	0.09^{b}
T_4	10^{c}	14^{b}	3^{b}	1.6^{b}	12^{c}	0.11^{c}	0.05^{c}
SEd	0.62	0.76	0.18	0.09	0.91	0.01	0.01
CV%	17.31	16.99	17.26	17.08	17.81	18.74	18.87
SE	2.18	2.70	0.64	0.32	3.20	0.04	0.019
CD at 5% level	1.31	1.62	0.39	0.19	1.92	0.02	0.01
	S	NS	NS	NS	S	S	S

Table 2. *Effect of different water regimes on nodulation and morphometric characters of Arachis hypogaea L inoculation with ARS2 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)
T_0	25.6^{a}	24^{a}	4^{a}	2.1^{a}	37^{a}	0.38^{a}	0.19^{a}
T_1	20^{b}	16.5^{c}	3.4^{ab}	1.7^{b}	2.5^{c}	0.3^{b}	0.14^{bc}
T_2	24.2^{a}	22^{a}	3.8^{a}	2^{a}	35^{a}	0.37^{a}	0.18^{a}
T_3	22^{a}	23^{a}	3.2^{b}	1.9^{a}	30^{b}	0.29^{b}	0.15^{b}
T_4	16^{c}	14.5^{c}	2.5^{c}	1.2^{c}	22^{c}	0.21^{c}	0.11^{c}
SEd	1.07	1.00	0.17	0.09	1.49	0.02	0.01
CV%	17.56	17.64	17.59	17.81	17.71	17.95	17.76
SE	3.78	3.52	0.59	0.31	5.27	0.05	0.027
CD at 5% level	2.27	2.12	0.36	0.19	3.17	0.03	0.02
	S	S	S	S	S	S	S

Table 3. *Effect of different water regimes on nodulation and morphometric characters of control plants of Arachis hypogaea L – 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)
T_0	16.5^{a}	19.2^{a}	4.1^{a}	2.1^{a}	52^{a}	0.25^{a}	0.13^{a}
T_1	12^{b}	14^{b}	3.8^{a}	1.9^{a}	38^{b}	0.24^{a}	0.13^{a}
T_2	16^{a}	18.5^{a}	4^{a}	2^{a}	45^{ab}	0.3^{a}	0.14^{a}
T_3	12^{b}	13^{bc}	3.8^{a}	1.9^{a}	30^{c}	0.2^{ac}	0.1^{b}
T_4	12^{b}	13^{bc}	3.8^{a}	1.9^{a}	30^{c}	0.2^{ab}	0.1^{b}
SEd	0.71	0.82	0.19	0.09	2.12	0.01	0.01
CV%	17.26	17.42	16.65	16.64	17.60	17.59	17.47
SE	2.50	2.88	0.65	0.32	7.50	0.04	0.02
CD at 5% level	1.50	1.73	0.39	0.20	4.50	0.03	0.01
	S	S	NS	NS	S	S	S

Table 4. *Effect of different water regimes on nodulation and morphometric characters of Arachis hypogaea L inoculation with ARS2 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)
T_0	26.8^{a}	21.2^{a}	4.5^{a}	2.3^{a}	1.11^{a}	0.42^{a}	0.21^{a}
T_1	18^{b}	15^{c}	3.8^{b}	1.8^{b}	100^{a}	0.39^{a}	0.2^{a}
T_2	26^{a}	20.5^{a}	4.5^{a}	2.3^{a}	100^{a}	0.42^{a}	0.21^{a}
T_3	24^{a}	19^{a}	4.5^{a}	2.2^{a}	70^{b}	0.35^{b}	0.2^{a}
T_4	18^{b}	14^{c}	3.5^{c}	1.8^{b}	50^{c}	0.27^{c}	0.13^{c}
SEd	1.12	0.89	0.20	0.10	4.48	0.02	0.01
CV%	17.47	17.49	17.16	17.12	18.37	17.67	17.77
SE	3.94	3.13	0.71	0.35	15.84	0.06	0.03
CD at 5% level	2.36	1.88	0.43	0.21	9.50	0.04	0.02
	S	S	NS	NS	S	S	S

Table 5. *Effect of different water regimes on nodulation and morphometric characters of control plants of Arachis hypogaea L – 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)
T_0	24.5^{a}	25.8^{a}	4.5^{a}	2.2^{a}	315^{a}	0.77^{a}	0.37^{a}
T_1	18^{bc}	18^{bc}	4.6^{a}	2.3^{a}	210^{bc}	0.61^{a}	0.3^{b}
T_2	26^{a}	26^{a}	4.8^{a}	2.4^{a}	300^{a}	0.74^{a}	0.37^{a}
T_3	25.3^{a}	25^{a}	5^{a}	2.4^{a}	240^{b}	0.68^{a}	0.34^{a}
T_4	20^{b}	21^{b}	4.3^{ab}	2.1^{ab}	150^{cd}	0.48^{c}	0.24^{c}
SEd	1.11	1.12	0.22	0.11	12.57	0.03	0.02
CV%	17.20	17.06	16.85	16.91	18.29	17.61	17.61
SE	3.91	3.95	0.78	0.38	44.44	0.11	0.05
CD at 5% level	2.35	2.37	0.47	0.23	26.65	0.07	0.03
	S	S	NS	NS	S	S	S

Table 6. *Effect of different water regimes on nodulation and morphometric characters of Arachis hypogaea L inoculation with ARS2 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)
T_0	42^{a}	26.4^{a}	4.6^{a}	2.3^{a}	370^{a}	1.25^{a}	0.71^{a}
T_1	46.5^{a}	24.5^{a}	4^{ab}	1.8^{b}	320^{ab}	0.89^{b}	0.45^{b}
T_2	46^{a}	25^{a}	4.8^{a}	2.4^{a}	355^{a}	1.24^{a}	0.68^{a}
T_3	27^{c}	19^{b}	4.5^{a}	2.3^{a}	300^{b}	0.94^{b}	0.52^{b}
T_4	23^{c}	15^{c}	4^{ab}	2^{ab}	220^{c}	0.77^{c}	0.41^{c}
SEd	1.94	1.11	0.21	0.10	15.71	0.05	0.03
CV%	18.60	17.90	16.95	16.95	17.74	17.75	17.87
SE	6.6	3.93	0.74	0.36	55.53	0.18	0.09
CD at 5% level	4.12	2.36	0.45	0.22	33.30	0.11	0.06
	S	S	NS	NS	S	S	S

Table 7. *Effect of different water regimes on nodulation and morphometric characters of control plants of Arachis hypogaea L – 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)
T_0	34.8^{a}	31^{a}	4.6^{a}	2.3^{a}	330^{a}	1.00^{a}	0.5^{a}
T_1	22^{b}	20.2^{b}	4.6^{a}	2.3^{a}	226^{b}	0.61^{b}	0.3^{b}
T_2	31^{a}	29.3^{a}	4.8^{a}	2.4^{a}	312^{a}	0.92^{a}	0.46^{a}
T_3	32.4^{a}	27^{ab}	5^{a}	2.5^{a}	261^{b}	0.88^{a}	0.43^{ab}
T_4	24^{b}	23^{b}	4^{ab}	2^{ab}	167^{c}	0.6^{b}	0.3^{b}
SEd	1.41	1.27	0.22	0.11	13.28	0.04	0.02
CV%	17.24	17.14	17.04	17.04	18.10	17.68	17.68
SE	4.97	4.47	0.78	0.39	46.93	0.14	0.07
CD at 5% level	2.98	2.68	0.47	0.24	28.15	0.09	0.04
	S	S	NS	NS	S	S	S

Table 8. *Effect of different water regimes on nodulation and morphometric characters of Arachis hypogaea L inoculation with ARS2 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)
T_0	44^{a}	34^{a}	4.6^{a}	2.3^{a}	378^{a}	1.6^{a}	0.92^{a}
T_1	46.5^{a}	24.5^{a}	4^{ab}	2^{ab}	320^{b}	1.14^{ab}	0.75^{b}
T_2	46^{a}	31.2^{a}	4.8^{a}	2.4^{a}	355^{a}	1.55^{a}	0.89^{a}
T_3	28^{b}	32.1^{a}	4.5^{a}	2.3^{a}	300^{b}	1.1^{ab}	0.7^{b}
T_4	23^{bc}	23.2^{b}	4^{ab}	2^{ab}	220^{c}	0.95^{b}	0.5^{c}
SEd	1.97	1.42	0.21	0.11	15.79	0.06	0.04
CV%	18.54	17.30	16.95	16.96	17.74	17.82	17.98
SE	6.95	6.01	0.74	0.37	55.82	0.22	0.13
CD at 5% level	4.17	3.01	0.45	0.22	33.47	0.14	0.08
	S	S	NS	NS	S	S	S

*T_0 – Control (-0.16 MPa) = field capacity, T_1 – (-0.24 MPa) = 2/3 f.c., T_2 – (-0.32 MPa) = 1/2 f.c., T_3 – (-0.49 MPa) = 1/3 f.c., T_4 – (-0.97 MPa) = 1/6 f.c. 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.

3.3. Estimation of Nitrogenase Activity

The nodule activities of *Arachis hypogaea* L plants were affected by water stress, this data were presented in Figure 1 and 2. Thus, ARA* followed a trend almost similar to activities observed under salt stress. However soil water depicts had more adverse effects on nitrogenase activities. T3 plants (-0.49MPa) of *Arachis hypogaea* L (inoculated) recorded about 60% of the nitrogenase activity of controls (field capacity). water stress severely affected nitrogenase activity of control stressed plants then Rhizobium inoculated stress plants.

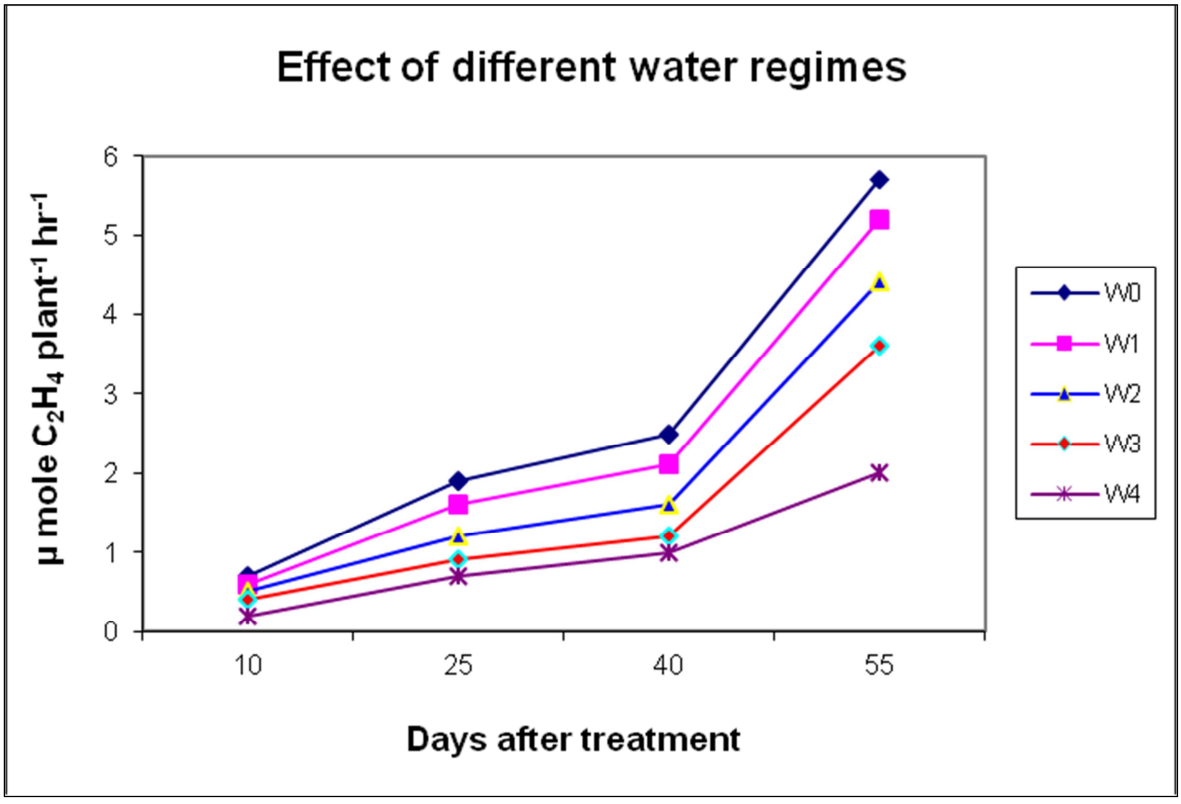

Figure 1. *Nitrogenase activity in control plants of Arachis hypogaea L.*

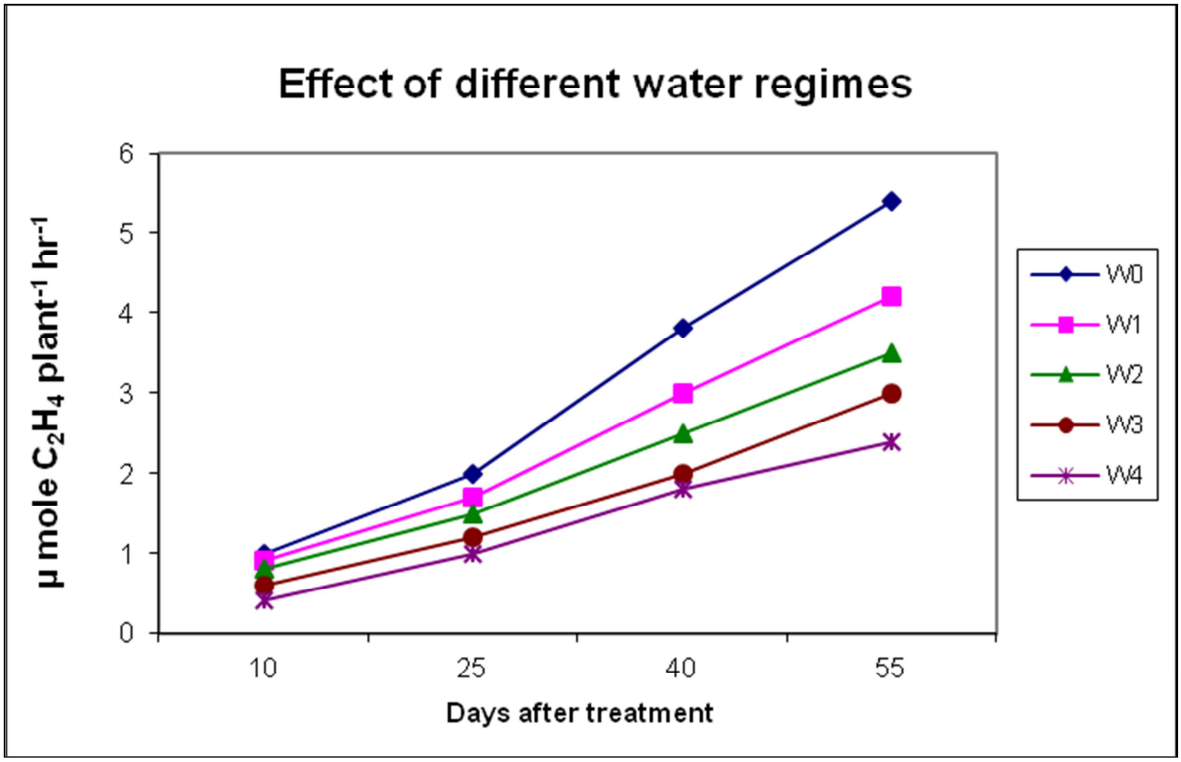

Figure 2. *Nitrogenase activity in Rhizobium inoculated plants of Arachishypogaea L.*

3.4. Determination of Leghaemoglobin Content

LHB considerably declined with decrease in soil water potential to give minimum values with T_4 treatment (-0.97 MPa). The increments of LHB* content with the sequential harvests of *Arachis hypogaea* L plants. At 76 day old plants (55 DAT) have more amount LHB content than 31 day old plants (10 DAT) of both varieties of leguminous plants (Figure 3 and 4).

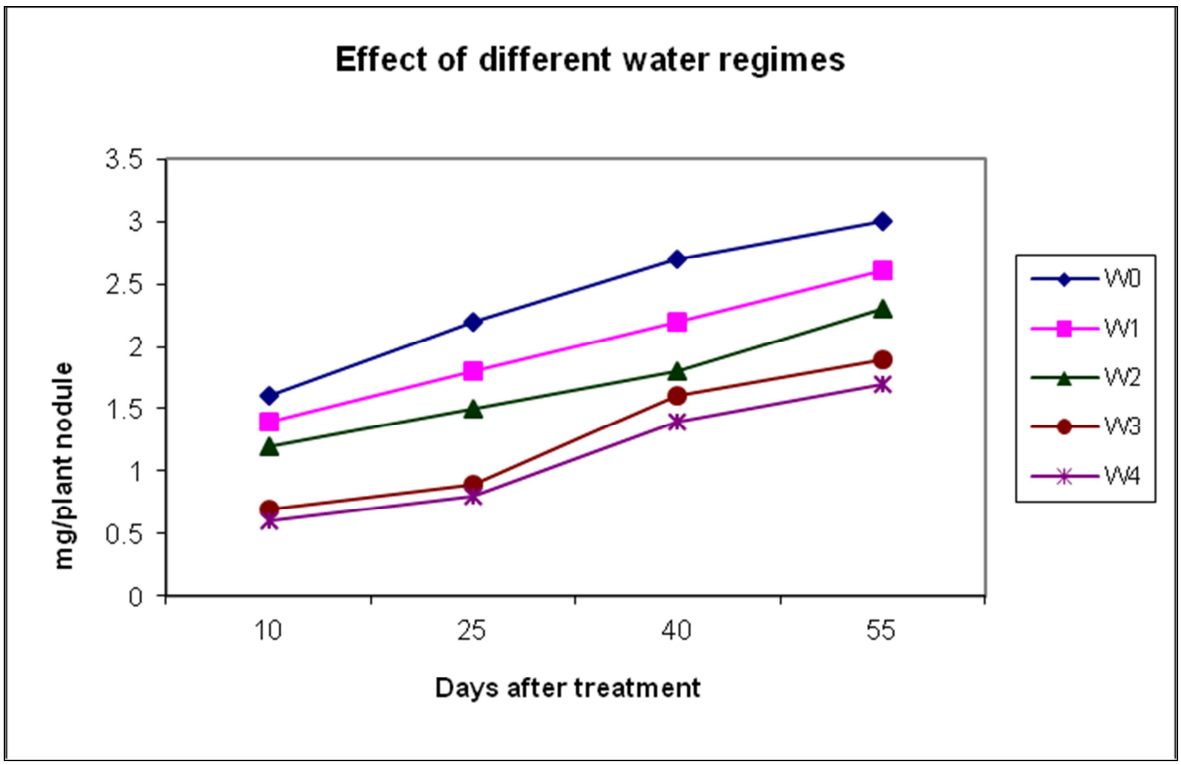

Figure 3. *Leghaemoglobin content in control plants of Arachis hypogaea L.*

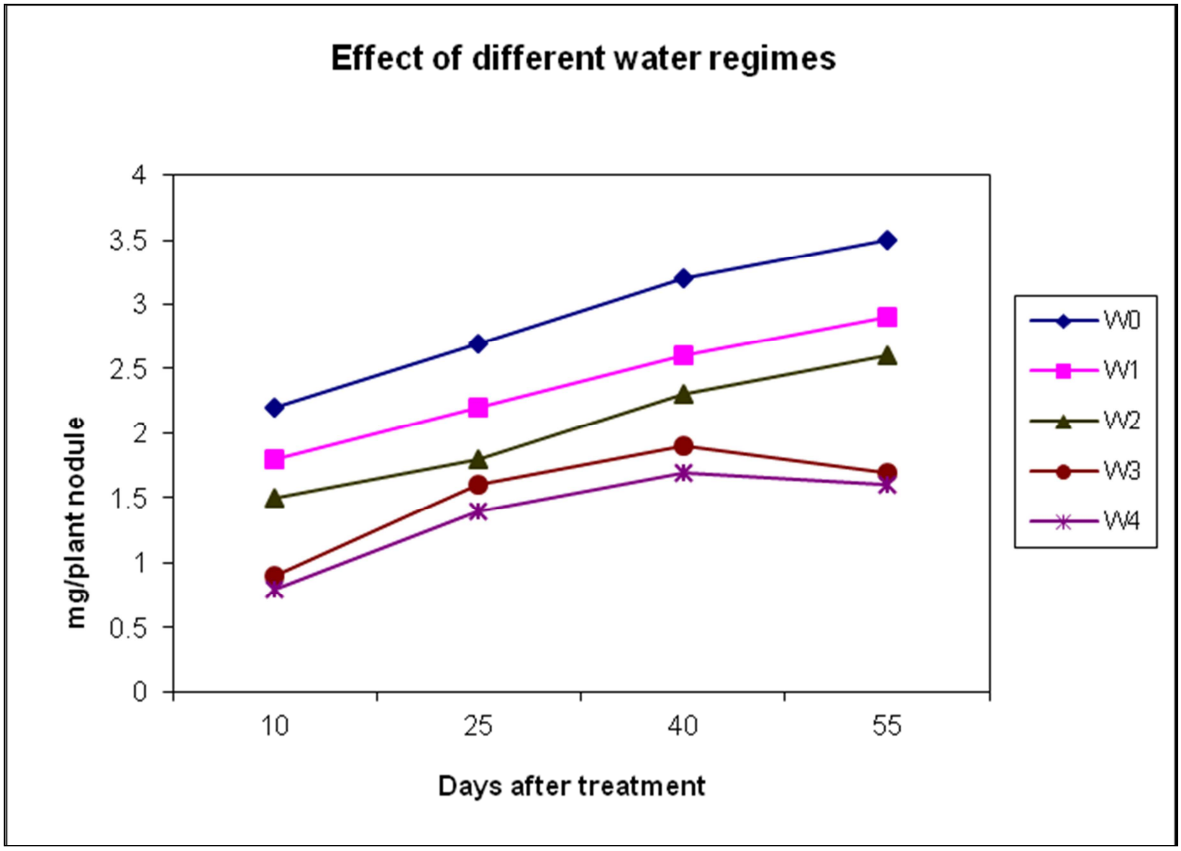

Figure 4. *Leghaemoglobin content in Rhizobium inoculated plants of Arachis hypogaea L.*

4. Discussion

Rhizobium isolated from nodules of Arachis hypogea plants. It is confirmed by morphological and colony characteristics and biochemical test.

In present investigation from the characterization tests, it is evident that all the isolates from *Arachis hypogaea* L plants are non ketolactose produces and showed no growth in Hofers medium. Pure Rhizobium isolates are unable to grow on lactose [14]. Rhizobium cannot grow at P^H 11 in the Hofers alkaline medium [15].

In leguminous plants, drought reduces nitrogen fixation and its related traits. The leguminous plants inoculated with rhizobium strain moderately resistant to drought stress then the control plants. The use of drought resistant cultivars should be of great advantage to retain high nitrogen fixation and acceptable yield. Drought is one of the major factor to reduce the growth and yield components of field crops.

The water stress decreasing the nodulation and plant growth parameters. However, the presence of more than 50 percent active nodules indicates that our experimental plants, *Arachis hypogaea* L are tolerant to mild water deficits. The nodules of inoculated plants yielded the greatest weight. Similar findings were made by [16] reported that the effects on different stages of N_2 fixation in cowpea plants. Drought stress reduces nodule number and nodule fresh and dry weight, but the reduction in these traits different among traits and between levels of drought stress. Several mechanisms have been suggested and reviewed to explain the varied physiological responses of several legumes when subjected to drought [17].

The local cultivars *Arachis hypogaea* L is more tolerant to water stress when inoculated with Rhizobium than the control plants. Moreover, the results obtained here indicate the Rhizobium is more suitable for cultivation of *Arachis hypogaea* L plant. The measurement of nitrogenase activity was based on the reduction of acetylene to ethylene as quantities by gas chromatography. Nitrogenase activity of both plant nodule Inoculated with rhizobial isolates showed maximum acetylene reduction than control stressed plants. The contribution of potential and reduction in the nitrogenase activity were different between drought and it was reduced with more severe stress [18].

The nodule number, weight and ARA^* of phaseolus vulgaris cultivars were significantly reduced at 50 percent soil FC. Nodulation and nitrogenase activity in both plant root were responded greatly to inoculation of Rhizobium. The presence of leghaemoglobin in legume nodules is considered as a prerequisite for N_2 fixation. Leghemoglobin is a nitrogen or oxygen carrier, it has a high affinity for oxygen and allows nitrogenase to function during N_2 fixation [17].

In the presence investigation, the highest LHB was recorded with plants that were given the basal nutrient solution without any added salts (control). Values of leghaemoglobin content slightly decreases at high solinity

levels. Also LHB considerably declined with decrease in water potential. The tendency of LBS content was to increase the plant growth (55DAT). The increase in the severity and duration of water stress affected the nitrogenase activity and leghaemoglobin content of nodules of chickpea plants [19].

From the present investigation, it may be concluded that the Rhizobial isolates showed better symbiotic performance with leguminous plants. Several environment conditions are limiting factors to the growth and activity of this N_2 fixing is strongly related to the physiological state of the host plant. Therefore, a competitive and persistent rhizobial strain is not expected to express it fully capacity for nitrogen fixation if limiting factors (eg. Salinity, unfavorable soil pH and temperature extremes). Impose limiting on the vigour of the host legume. The Rhozobial isolates showed tolerant to salt and drought stress than the control and fix high level of N.

5. Conclusion

From the present investigation, concluded that the *Arachis hypogaea* L plant which are affected or induced with nitrogen fixing bacteria such as Rhizobium is resistant towards the drought condition (water stress) and have greater ability to survive and give good yield, while the control plant during water stress condition gives very low yield.

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