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Role of *Rhizobium leguminosarum* In Mitigating the Inhibitory Effects of Water Deficit on *Arachis hypogaea*

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ABSTRACT

The current study investigated the role of inoculating groundnut with isolated *Rhizobium leguminosarum* under water stress. Three levels of irrigation {80% (control), 60% (D1), and 40% (D2) of field capacity} were employed in the presence or absence of *Rhizobium*. Results revealed that plant growth biomarkers and physiological attributes were altered under drought conditions, while *Rhizobium*-groundnut-symbiosis with or without water stress showed a remarkable improvement in plant growth (branch number, shoot and root fresh and dry weights) photosyntheticpigments and the quantum yield of PSII (Fv/Fm) as well as the increase in nodules' weight, number, leghaemoglobin, and N_2 contents. The electrograms displayed quantitative and qualitative characteristic changes among the D1 and D2 separately or with *Rhizobium* inoculation. Drought-induced synthesis of high or low MWs new stress protein bands with low intensities; additionally, *Rhizobium* inoculation resulted in the increase in the number of bands, besides the elucidation of the relationships among different treatments as showed by dendrogram cluster analysis. The overall results suggest that *Rhizobium*-symbiosis regarded as a promising and effective tool for the modulation of groundnut to conquer water deficiency.

INTRODUCTION

Global warming and climatic changes alter the rainfall pattern as a result of increasing industrialization, urbanization, and other anthropogenic activities, the major causes of drastic abiotic stresses that are diminishing crop production all over the world. Drought stress is considered to be the most prominent hazard; it affected about 33% of land area which contributes to the decline in agricultural productivity (Hasanuzzaman *et al.,* 2020). Water scarcity affects plants′ growth and development via distraction of the plant′s physiological processes and metabolic activities causing yield loss and inciting food crises unanimously (Chandra *et al.,* 2021).

Arachis hypogaea L. is considered one of the imperative oily seed crop production, it has great economic importance worldwide with production reaching more than 50M tons in 2018 (FAO, 2021). It is mostly cultivated in different regions of the world under rain-fed conditions (Patel *et al.,* 2020).

Its production endures a loss of around 6M tons on account of drought stress (Bhatnagar-Mathur *et al.,* 2014), consequently, it is necessary to develop a new technique for improving crop performance under water shortage via the alteration in morphological and physio-biochemical mechanisms. Several strategies are followed to alleviate drought stress effects; one potential strategy is the biological interaction with plant growth-promoting bacteria (PGPB) which was found to have a prospective role in promoting plant tolerance (Gupta *et al.*, 2022). Rhizobia are soil bacteria that have the ability for legumes symbiotic involvement; they can inhabit and fix atmospheric nitrogen within the root or stem nodules. Rhizobia-legumes symbiosis regards as an environmentally and economically necessary agricultural performance, employed in legumes production (Goyal *et al.*, 2021).

Agricultural sustainability entails lessening the production and applications of mineral fertilizers, which will augment and necessitate legume-based production (Papendiek*et al.,* 2016). The rhizobia-legume symbiotic association is a key provider of nitrogen nutrition for plants along with they provide energy and carbon for rhizobia. Symbiosis is sturdily related to the physiological state of the host plant, it relies on the *Rhizobium* strain, the legume genotype, and their interactions with the bio-physical environment (Muktadir*et al.*, 2020; Allito *et al.,* 2021). Several studies demonstrated that the application of various strains of PGPB can promote and improve plants stressed environmentally by drought through physiological and biochemical changes (Gouda *et al.,* 2018; Goyal *et al.*,2021). *Rhizobium leguminosarum* potentially contributes to limiting the impact of drought stress; however, the effect of different rhizobia strains was different with *Vicia faba* genotypes (Amine-Khodja *et al.,* 2022). The aim of the current investigation was to evaluate the performance of selected *Rhizobium* strains from several isolated ones on some growth biomarkers and physiological characteristics of *Arachis hypogaea*plants under water deficit conditions.

MATERIALS AND METHODS

Collection, Isolation and Culture of Groundnut Nodule Rhizobia:

The tested rhizobia strains were collected from different areas in El-Beheira Governorate. Isolates and their locations are listed in Table 1. The roots′ groundnut nodules were separated, and washed several times with sterile distilled water; vortexed in 0.05% (v/v) Tween 20 to remove adhering particles of soil, nodules were surface sterilized by soaking in ethanol for 30s, immersed in sodium hypochlorite solution (1%), and rinsed several times with autoclaved distilled water. Nodules were crushed with a plastic pestle with 40 µl of water. The nodule suspension was inoculated (15µl) onto plates with yeast extract mannitol salt broth medium {mannitol (10g), K_2HPO_4 (0.5g), yeast extract (0.5g), $MgSO4.7H_2O$ (0.2g), NaCl (0.1g), distilled water 1000ml} the pH was adjusted to 6.8 and the plates were incubated at 28˚C for 3–5 days. The isolates with different colony morphologies were selected and stored in 30% glycerol at −80˚C (Vincent, 1970).

Table 1: Origin of isolates and soil characteristics.

Isolate code	Origin	Location	Number of bacterial nodules plants^{-3}	Soil type	pH
Rh1	Monophya, Behira, Egypt	30°58'0.9"N 32°26'30"E	6	Clay	7.0
Rh2	Markzbadr, Behira, Egypt	30°35'25.0"N 30°43'29.9"E	7	Sandy loam	8.0
Rh3	Komhamada, Behira, Egypt	30°40'21.0"N 30°47'08.2"E	15	Sandy loam	7.85
Rh4	Itay El Barud, Behira, Egypt	30°87'81.7"N 30°67'42.4"E	5	Clay	7.5

Early Plant Growth Promotion by Rhizobia Strains:

The bacterial isolates were initially screened based on their ability to promote the early plant growth of groundnut to select the best strain for the main experiment. Fourrhizobia strains (Rh1, Rh2, Rh3 and Rh4), with the recommended concentration of 1.3×10^6 viable cells/ml were added, stirring with *Arachis hypogaea* seeds immediately before planting. Inoculated seeds as well as un-inoculated ones (control) were planted in the pots filled with sterilized soil mixture and incubated in a greenhouse for 30 days. At the end of the experimental period, nodules were carefully collected to detect the number, plus the shoots and roots-dry weights.

Experimental Design:

Three randomized design blocks and a factorial scheme 2x3, with one cultivar, one effective *Rhizobium* strain and three levels of water stress were employed. Based on the plant growth promoting ability, *Rhizobium (Rhizobium leguminosarum* bv. *phaseoli* - strain BR 353) strain was chosen for further experiments. A greenhouse experiment was conducted at the Faculty of Agriculture (Saba Basha) at Alexandria University, Egypt, from April–July 2021. Seeds of *Arachis hypogaea*L. (Giza 6 cultivar) were purchased from the Horticulture Research Center, Agricultural Research Center (ARC), Ministry of Agriculture, Behera, Egypt. Seeds were surface sterilized with sodium hypochlorite solution (0.1%) for 5 min, then washed with distilled water several times. In sterilized Petri dishes seeds were germinated for two days, thereafter, implanted in pots (14cm x 22cm) containing sterilized soil mixture inoculated with 1mL of a mixture of the effective strain of *Rhizobium leguminosarum*. The physicochemical characteristics of the soil were summarized in Table 1. Pots (in triplicates) were irrigated every two days intervals with distilled water at field capacity for 15 days before drought stress started. Drought stress treatments were imposed *viz*, 80%, 60% (D1) and 40% (D2) of the field capacity, these treatments reflecting conditions achieved as the optimum level of water supply (control plants) and drought stress, respectively. Pots were irrigated with sterilized water every two-day interval throughout the whole experimental period. After 20 days, homologous plants were harvested, washed thoroughly from adhering soil particles, gently plotted, dissected to shoots and roots and quickly saved for estimation of the various growth parameters and chemical analyses. Roots with nodules were washed with tap water gently and blotted with filter paper for drying, nodules' number per plant was determined by collecting and counting all nodules on each of the plants and computing the average.

Plant Growth, Nodulation, Nitrogen Content and Nodules′ Leghaemoglobin Content:

Plants were harvested after 65 days of germination, separated into aboveground shoots and roots, carefully rinsed with distilled water and dried at 65°C for 72 hours before determining the dry weight. The fresh weight (FW), dry weight (DW), shoot and root lengths, nodule fresh and dry weights and the number of nodules were

counted. Total nitrogen of the soil samples (randomly collected from the surface to 25cm in depth, air-dried and passing through a 2mm sieve), shoots, roots and nodules was determined by the colorimetric method of Linder (1944) using Nessler´s reagent following digestion in a mixture of concentrated sulphuric acid and perchloric acid.

Nodules′ leghaemoglobin were detected following the method described by Wilson and Reisenauer (1963), nodules (0.5g) were homogenized in aliquots of Drabkin's reagent (10ml) and leghaemoglobin was quantified spectrophotometrically at $A_{540,}$ bovinhaemoglobin was used as a standard, and values are expressed as mg g^{-1} nod. FW.

Photosynthetic Pigments and Quantum Yield of PSII:

The photosynthetic pigments were determined according to methods described by Moran (1982) using N, N-dimethyl formamide (DMF), and total carotenoids content was calculated according to Wellburn (1994) and related to leaf fresh weight. Measurement of chlorophyll fluorescence was performed with an OS-30P pulse-modulated chlorophyll fluorometer (Opti-sciences, Hudson, and USA) following the procedure described by Van Kooten and Snel (1990).

Protein Extraction and Gel Electrophoresis:

For sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), shoot tissues of each treatment were ground to powder under liquid nitrogen and melted in ice-cold extraction buffer (50mM NaH_2PO_4, pH 7; 10mM EDTA, pH 8; 10 mM β-mercaptoethanol; 0.2% Triton X-100) per g of tissue, followed by centrifugation at 14,000 rpm, 4°C for 15min. The supernatant was used for SDS-PAGE. The protein quantification was done according to the method given by Bradford (1976). Electrophoresis was performed on 12.5% SDS-gel Laemmli (1970). Gels were stained in 0.5% Coomassie Brilliant Blue R250 in ethanol and 10% 3-chloroacetic acid. A gel documentation system (Geldoc-it, UVP, England), was applied to analyze the banding pattern, molecular mass and band percentage using Totallab analysis software (ww.totallab.com, Ver.1.0.1).

Statistical Analysis:

Statistical analysis of the results was carried out according to Duncan's multiple-range tests using SPSS-20. Data were subjected to one-way ANOVA following the method of Sokal and Rohlf (1995). Differences between treatment means were considered statistically significant at $p \leq 0.05$.

RESULTS

Early Plant Growth Promotion by Rhizobia Strains:

In order to assess the most effective strain from the four isolated ones, the plants were uprooted and observed for shoot and root dry weights and nodule numbers. Based on the results (Table 2) The four isolates namely *Rhizobium mesosinicum* strain R1-99 (Rh1), *Rhizobium pusense strain* A5 (Rh2), *Rhizobium leguminosarum* bv. *phaseoli* - strain BR 353 (Rh3) and *Rhizobium giardinii* bv. *giardinii* strain H152 (Rh4) confirmed their ability to nodulate groundnut by the formation of typically reddish nodules. Rh3 showed the highest mean values of, shoot and root dry weights and nodule numbers.

Table 2: Variations in number of nodules and nodules' dry weights with isolated rhizobial strains.

Strains	Nodule number plant^{-1}	Dry weight (g plant^{-1})	
		Shoot	Root
Control	0±0.0	0.33±0.02	0.16±0.01
Rh1	10±0.21	0.76±0.03	0.26±0.01
Rh2	13±0.31	0.92±0.06	0.27±0.02
Rh3	30±0.62	1.49±0.08	0.43±0.02
Rh4	10±0.23	0.72±0.028	0.21±0.01

Plant Growth, Nodulation, Nitrogen Content and Nodules LeghaemoglobinContent:

The effects of *Rhizobium* inoculation on the growth biomarkers and physiological characteristics of groundnut plants were analyzed. *Arachis hypogaea* plants were grown under moderate (D1) and severe (D2) water stress (80%, 60% and 40%) of the field capacity in the presence or absence of *Rhizobium leguminosarum*. Data represented in Figure 1 showed that no nodule was observed in uninoculated roots (Fig. 1A and B). Active nodules pink in color were observed in the roots of the plants inoculated with *Rhizobium* (Fig. 1C). Data represented in Figure 1 illustrated that the groundnut plants inoculated with *Rhizobium*, recorded a significant ($P \leq 0.05$) increase in all plant growth parameters compared with un-inoculated plants in the normal irrigation (control plants) or drought treatment. In general, plants were negatively affected by water shortage, the reduction in the number of branches reached 15% and 22% under D1 and D2 treatments, respectively in the un-inoculated plant. The reduction values for shoot height, fresh and dry weight reached 32%, 41% and 28%, respectively under D2 treatment, conversely, values for root length and dry weight showed an increase of about 8% and 6% compared with the control (Fig. 2B).

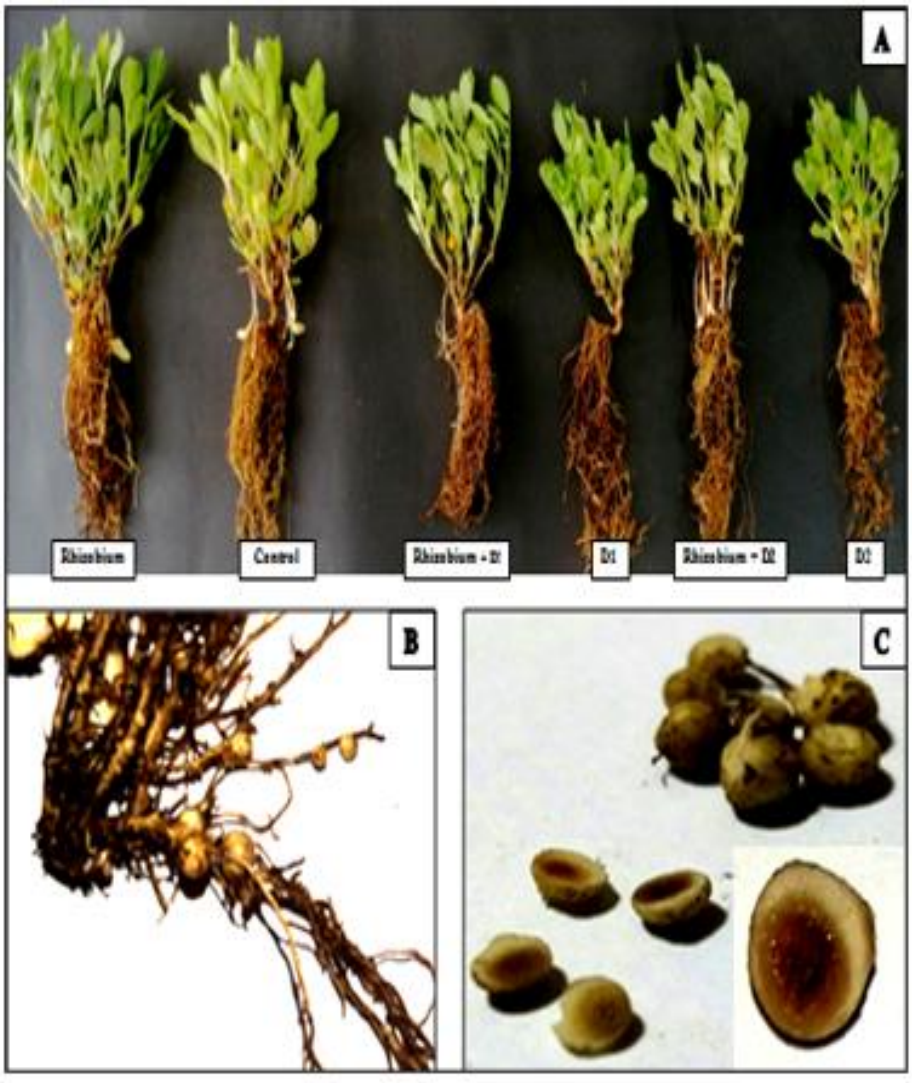

Fig. 1: Effect of *Rhizobium leguminosarum* inoculation and water stress on growth of 65-day old groundnut plants (A): performance, (B): roots with nodules, and (C): active nodules.

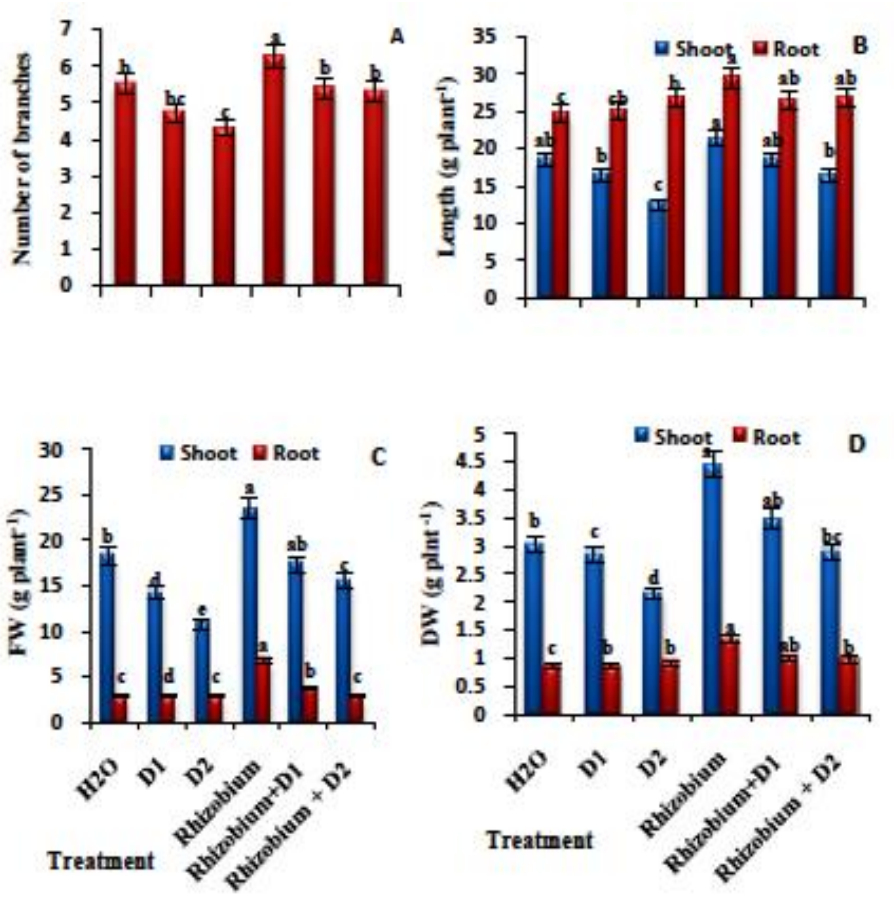

Fig 2: Effect of *Rhizobium leguminosarum* inoculation and water stress on (A): branches' number, shoot and root (B): lengths, and (C,D): fresh and dry weights of 65-day old groundnut plants. Different letters indicate significant difference by Duncan's multiple range tests($p \leq 0.05$). Values are means ±SE (n= 4).

Rhizobium inoculation under water deficit resulted in a significant ($p \leq 0.05$) increase in nodule number, nodule fresh, dry weight and nitrogen content relative to inoculated non-stressed controls (Fig. 3).The increase values were 1.2- and 1.5-fold nodule number per plant, 13% and 47% nodule FW, 24% and 64% nodule DW, and N content were increased by about 5% and 22% under D1 and D2 treatments respectively. It can be seen from the data represented in Figure 3Dthat the interaction of D1 and D2 water deficit and *Rhizobium* inoculation significantly ($P \leq 0.05$) increased the nodules leghaemoglobin by about 1- and 1.3-fold per plant as compared to inoculated-unstressed ones. According to the results (Fig. 3E), drought significantly reduced nitrogen content by 7% for shoots and roots under D1-treatment, and 14% and 18% under D2-treatment. Shoots and roots-N was increased by 1.5-fold in inoculated-unstressed plants in comparison with control un-inoculated ones. The corresponding values for inoculated stressed were 1.3- 1.4-fold for shoots and roots under D1 and 1.1- and 1.5-fold for D2 treatments compared with the un-inoculated plants which were not exposed to stress.

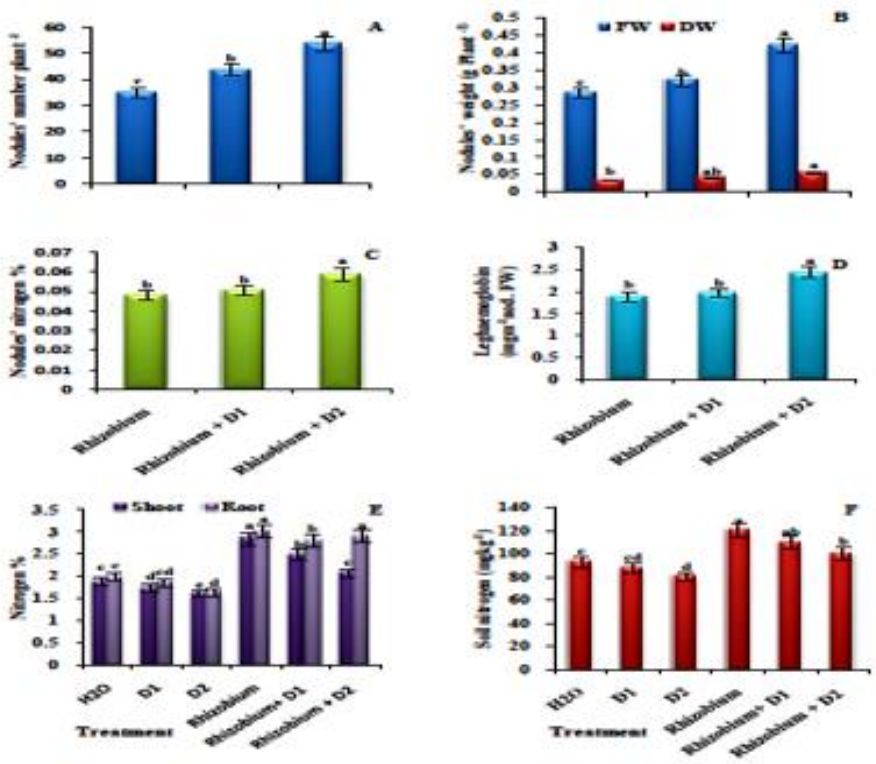

Fig 3: Effect of *Rhizobium leguminosarum* inoculation and water stress on (A): nodules' number, (B): nodules' weight, (C): nodules' nitrogen %, (D): nodules' leghaemoglobin, (E): shoot and root nitrogen %, and (F): soil nitrogen % of 65-day old groundnut plants. Different letters indicate significant difference by Duncan's multiple range tests($p \leq 0.05$). Values are means ±SE (n= 4).

Photosynthetic Pigments and Maximum Quantum Efficiency of PSII Photochemistry

Leaf chlorophyll content (Chl. a and b, and total Chl.) were also affected by drought stress; the reduction values in total Chl. reached 20% and 42% under D1 and D2 water deficit treatments in comparison with the control plants (Figure 4). Otherwise, carotenoids content increases significantly with water deficit treatments. The impact of drought and *Rhizobium* inoculation on leaf photosynthetic pigments was evaluated (Figure 4). The highest values recorded in inoculated-unstressed plants reached about 45%, 49% and 46% in Chl. a, b, and total Chl., respectively.

The corresponding values under the interaction treatments reached about 14%, 17%, and 15% for D1 treatment and 11%, 10% and 10% for D2 treatment, respectively compared with control un-inoculated plants. Carotenoids were insignificantly affected under combined treatments; values were nearly similar to the controls. The study of PSIIphotochemistry results showed that D1 and D2 water regimes induced minimum F_o, Fv, Fm, Fv/F_o, and Fv/Fm. There was a marked decrease in Fv/Fm of water-stressed plants compared to the control; the reduction percentage was about 7% and 13% respectively compared with the control (Figure 4). Bacterial inoculation significantly diminished the adverse effect of drought stress on groundnut plants, the Fv/Fm value of inoculated-unstressed plants reached about 1-fold higher than that of the control, where the corresponding values of inoculated and stressed ones showed no significant differences in comparison with control, but significantly higher than that of un-inoculated- stressed with the values reached 8% and 10% higher in comparison with D1 and D2 respectively (Fig. 4).

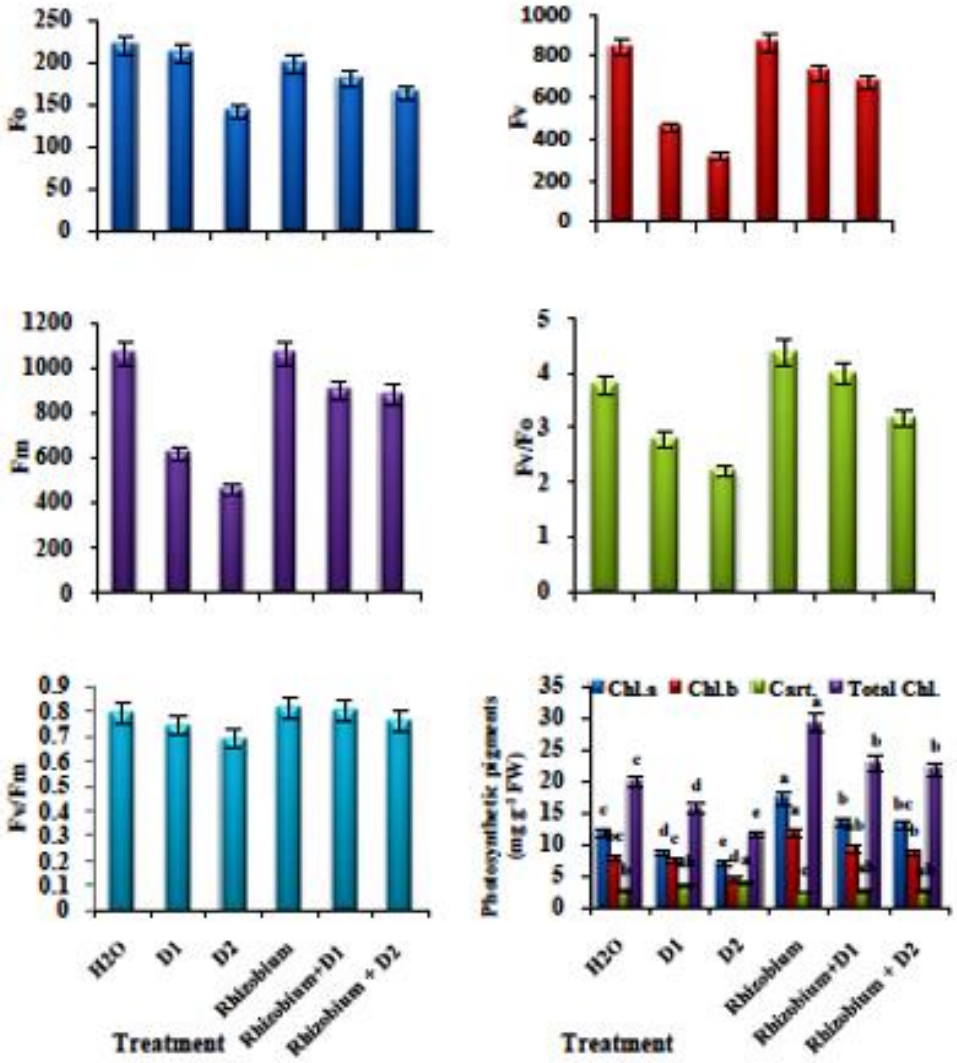

Fig 4: Effect of *Rhizobium leguminosarum* inoculation and water stress on photosynthetic pigments and photosynthetic efficiency of 65-day old groundnut leaves. Different letters indicate significant difference by Duncan's multiple range tests ($p \leq 0.05$). Values are means ±SE (n= 4)..

Changes in Protein Profiles:

Total protein was extracted from shoots and roots, separated by SDS-PAGE and the protein profiles revealed major differences among all treatments. The electrograms exhibited distinctive quantitative and qualitative alterations among the D1 and D2 with or without *Rhizobium* inoculation compared with the control. As shown in Figure 5, 6 and Table 3, protein alterations were detected based on changes in polypeptides' molecular weights (MWs), band intensities, fractionation of some bands, and the appearance of new polypeptides and disappearance of some others.

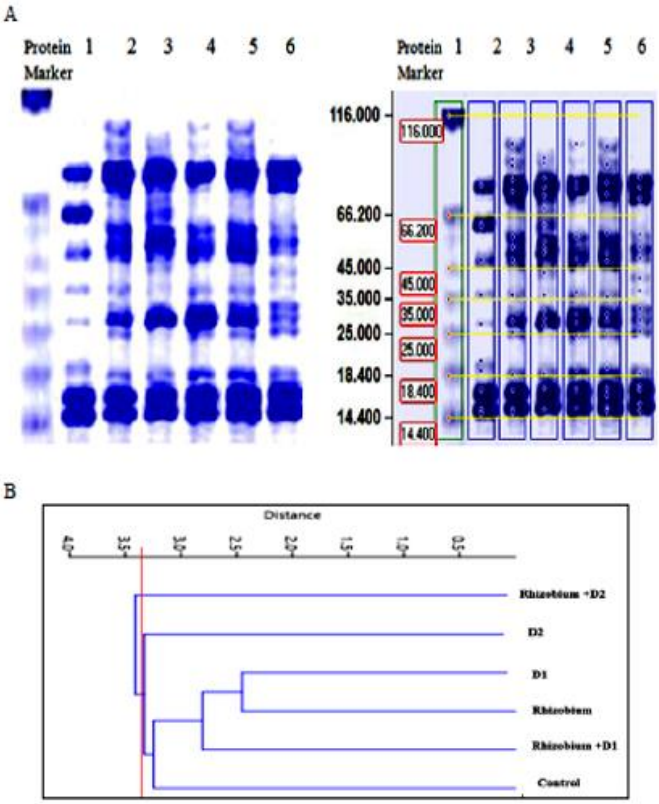

Fig 5:(A) SDS-PAGE protein electrophoretic patterns and (B) Phyllogenetic tree based on protein fingerprinting patterns for 65-day old groundnut shoots under *Rhizobium leguminosarum* inoculation and water stress. 1: Control, 2: D1, 3: D2, 4: *Rhizobium*, 5: *Rhizobium*+D1 and 6: *Rhizobium*+D2.

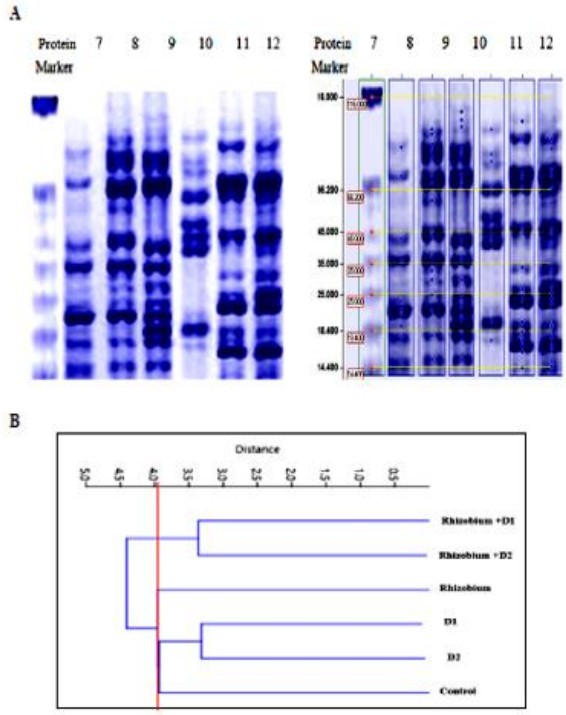

Fig. 6: (A) SDS-PAGE protein electrophoretic patterns and (B) Phyllogenetic tree based on protein fingerprinting patterns for 65-day old groundnut roots under *Rhizobium leguminosarum* inoculation and water stress. 7: Control, 8: D1, 9: D2, 10: *Rhizobium*, 11: *Rhizobium*+ D1 and 12: *Rhizobium* + D2.

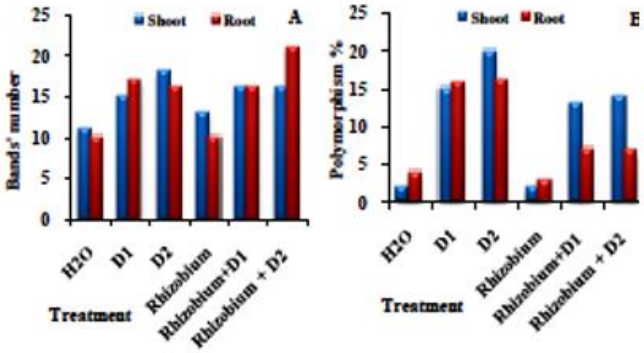

Fig 7: Bands' number (A) and % polymorphism of electrophoretic protein patterns (B) of 65-day old groundnut shoots and roots under *Rhizobium leguminosarum* inoculation and water stress.

Based on the electrophoretic pattern, polymorphism was high in roots than in shoots, but in the presence of *Rhizobium* it decreased and the decline was remarked more in roots than in shoots (Fig. 7B). The total number of bands detected for the control root and shoot was 10 and 11 bands with MWs ranging nearly between 87.5-14.66 KD and 79.96-14.77 KD respectively. Remarkably from our results the drought stress-induced synthesis of new stress protein bands where these unique bands (high or low MW) had low intensity. Roots of D1 and D2 treatments showed 17, and 16 bands respectively with 7 and 6 excess bands than their controls. Similarly, shoots of D1 and D2 displayed 15 and 18 bands individually with 4 and 7 excess bands than their control (Fig. 7A). Application of *Rhizobium* showed the disappearance of one polypeptide in the root of D1 treatment (103.5 KD) and two polypeptides in the shoot of D2 treatment (94.016 KD and 91.151 KD), meanwhile, five polypeptides newly appeared in D2-treated root (77.41 KD, 58.66 KD, 16.31 KD, 14.99 KD, 14.52 KD and one in D1-treated shoot (30.26KD). Obviously, roots and shoots before and after *Rhizobium* inoculation shared 4 and 6 bands. The shared four root bands exist at a range of 66.2-68.2 KD, 42.6-46.94 KD, 19.18-21.32 KD and 17.12-17.69 KD while the six common shoot bands are at 81.34-84.11KD, 75.92-79.29 KD, 43.56-48.49KD, 27.09-28.63 KD, 16.95-17.95 KD, and 15.14-15.64 KD (Table 3).

Table 3: Computerized analysis for SDS-PAGE electrophoretic protein patterns of 65-day old groundnut shoots grown under *Rhizobium leguminosarum* inoculation and water stress.

Control				D1 (60% water deficit)			D2 (40% water deficit)			*Rhizobium*			*Rhizobium* + D1			*Rhizobium* + D2		
Band No	Band %	MW	Rf	Band %	MW	Rf	Band %	MW	Rf	Band %	MW	Rf	Band %	MW	Rf	Band %	MW	Rf
1	18.36	79.96	0.250	4.35	101	0.125	0.99	94.016	0.167	1.1	101.27	0.125	2.21	99.81	0.133	3.66	84.11	0.225
2	11.04	62.51	0.362	0.90	93.30	0.171	1.31	91.151	0.183	8.91	81.34	0.242	2.29	90.44	0.188	19.61	79.29	0.254
3	8.93	61.31	0.371	3.32	89.73	0.192	2.12	83.408	0.229	10.81	78.61	0.258	14.22	81.34	0.242	8.66	73.92	0.287
4	8.41	47.98	0.471	4.72	82.72	0.232	11.93	79.285	0.254	7.07	56.63	0.404	7.88	75.92	0.275	4.34	57.78	0.396
5	2.47	36.32	0.575	20.74	77.25	0.267	7.56	75.245	0.279	14.84	48.49	0.467	2.52	58.36	0.392	8.36	52.73	0.433
6	1.86	27.46	0.662	9.04	56.64	0.404	0.86	69.364	0.317	3.66	37.20	0.567	10.53	50.58	0.450	3.12	48.49	0.467
7	0.83	20.26	0.771	1.44	52.73	0.433	8.90	61.906	0.367	6.78	30.26	0.633	10.90	47.47	0.475	2.81	41.69	0.525
8	5.43	19.03	0.800	12.72	47.98	0.471	6.11	54.935	0.417	14.6	27.84	0.658	3.65	35.88	0.579	1.34	37.64	0.563
9	15.90	16.95	0.858	3.17	36.32	0.575	5.31	52.185	0.438	3.06	24.68	0.696	4.97	30.26	0.633	4.80	31.95	0.617
10	15.21	15.14	0.917	10.63	28.23	0.654	12.42	50.581	0.450	6.13	19.19	0.796	8.55	28.23	0.657	5.13	28.63	0.650
11	11.56	14.77	0.929	2.52	25.32	0.688	2.24	43.563	0.508	5.37	17.09	0.854	2.91	25.00	0.692	4.98	25.66	0.683
12				3.43	18.56	0.813	1.79	36.318	0.575	3.61	16.81	0.862	6.21	19.37	0.792	3.97	18.71	0.808
13				10.52	16.95	0.858	2.63	31.096	0.625	14.08	15.27	0.912	5.49	17.09	0.854	4.20	17.95	0.846
14				8.51	15.14	0.917	4.91	29.027	0.646				5.69	16.42	0.875	4.59	16.95	0.858
15				4.00	14.89	0.925	9.53	27.089	0.667				6.60	15.39	0.908	9.09	15.64	0.900
16							3.41	18.711	0.808				5.37	15.02	0.921	11.32	15.02	0.921
17							11.42	17.092	0.854									
18							6.57	15.142	0.917									

Table 3 continued. Computerized analysis for SDS-PAGE electrophoretic protein patterns of 65-day old groundnut roots grown under *Rhizobium leguminosarum* inoculation and water stress.

Control				D1 (60% water deficit)			D2 (40% water deficit)			*Rhizobium*			*Rhizobium* + D1			*Rhizobium* + D2		
Band No	Band %	MW	Rf	Band %	MW	Rf	Band %	MW	Rf	Band %	MW	Rf	Band %	MW	Rf	Band %	MW	Rf
1	11.60	87.50	0.241	6.93	98.77	0.172	2.08	107.13	0.120	2.81	99.12	0.169	0.82	103.5	0.141	4.53	94.08	0.202
2	11.95	73.79	0.325	2.47	95.83	0.190	1.39	102.70	0.148	4.03	85.81	0.254	0.08	94.08	0.202	1.33	89.00	0.234
3	9.99	43.18	0.542	1.54	90.93	0.220	2.69	99.26	0.169	6.08	81.94	0.278	11.79	75.46	0.319	8.92	77.41	0.306
4	11.79	36.63	0.617	7.09	86.03	0.250	5.66	87.02	0.244	2.91	68.86	0.359	2.22	72.03	0.335	7.77	74.15	0.327
5	5.17	23.59	0.756	5.61	83.11	0.268	7.47	83.60	0.265	13.42	66.20	0.375	9.90	68.20	0.363	6.16	67.54	0.367
6	9.37	21.66	0.786	2.56	74.38	0.322	4.91	72.93	0.331	17.86	51.93	0.464	16.21	46.43	0.504	3.10	58.66	0.419
7	10.11	20.84	0.801	9.90	67.16	0.367	5.75	71.04	0.343	17.64	46.94	0.500	4.89	33.86	0.637	10.52	46.44	0.504
8	5.32	18.85	0.846	9.44	59.57	0.416	1.73	66.68	0.370	21.69	41.69	0.548	4.42	26.75	0.710	4.43	44.11	0.524
9	12.81	17.85	0.873	4.45	45.98	0.515	18.92	42.60	0.548	10.53	19.518	0.823	3.98	25.00	0.730	4.11	40.944	0.556
10	11.88	14.66	0.976	7.33	43.76	0.536	5.76	36.63	0.617	3.53	17.35	0.883	11.42	23.48	0.750	7.85	32.68	0.649
11				7.53	37.15	0.611	10.56	29.87	0.687				7.33	19.52	0.823	8.04	27.50	0.702
12				5.05	32.94	0.657	3.78	24.75	0.741				3.69	18.25	0.865	1.58	25.67	0.722
13				5.02	29.55	0.690	8.03	22.77	0.768				3.28	17.12	0.887	5.48	24.06	0.742
14				2.33	24.27	0.747	7.05	19.98	0.819				4.32	16.59	0.903	4.47	22.93	0.758
15				11.52	21.32	0.792	7.92	17.75	0.877				4.90	15.96	0.923	4.56	19.18	0.831
16				5.03	17.64	0.880	6.33	15.28	0.955				2.44	14.40	0.976	2.70	18.86	0.839
17				6.17	15.19	0.958										2.42	17.55	0.875
18																2.49	16.73	0/899
19																3.54	16.31	0.915
20																2.12	14.99	0.956
21																3.82	14.52	0.972

Dendrogram cluster analysis of groundnut proteins profile resulted from SDS-PAGE (Figure 5B and 6B) elucidated the relationships among different treatments, three groups were discriminated at a similarity distance of 3.9 for roots and 3.3 for shoots. The first group in root treatments clustering comprised both samples treated D1 and D2 with the control. In the other two groups, *Rhizobium* separated either alone or as a mono-clade combined with D1 and D2 from the rest of the samples. While in shoots clustering both samples treated D1 and D1 with *Rhizobium* with the control in a group and the D2 treatments and D2 with *Rhizobium* in the other two groups.

DISCUSSION

The affiliated intent of this study was to assess the effect of the best strain from several isolated rhizobial strains on some morphological and physiological characteristics of groundnut under different water deficit regimes {80% (control), 60% (D1) and 40% (D2)} of water field capacity. A preliminary screening was conducted for ten isolated rhizobacteria, from different areas of groundnut fields in El-Beheira Governorate, four *Rhizobium* strains inoculation were studied for growth-promoting attributes under greenhouse conditions. The inoculation experiments described herein revealed that the response of the tested groundnut cultivar varied with the four *Rhizobium* strains. *Rhizobium leguminosarum* bv (Rh3) strain was selected for further study as itperformed better regarding significant differences ($p \leq 0.05$) in the dry weights of shoot and root and nodule number plant^{-1}. This result corroborated former reports that high nodulation was obtained from groundnut when appropriate symbiotic partners were supplied throughout the compatibility between the groundnut variety and *Rhizobium* strains (Khalid *et al.,* 2020).

Results revealed that drought stress (D1 and D2) significantly decreased mean values of branch number, lengths of shoots and roots, fresh and dry weights, and the content of leaf chlorophylls as well as the quantum yield of PSII (Fv/Fm). The upshot of drought stress depends on its duration, intensity, and plant growth stage. Our findings are further supported by Nawaz *et al.* (2020) and Schumacher *et al.* (2021) on maize and potato plants. According to the results obtained, the carotenoids content increased in response to 60% and 40% of water stress in comparison with 80% water field capacity; thereby the increasing level may be due to their protective role in photosynthesis; photo-protection, and their

antioxidant activity from water shortage (Zhang *et al.*, 2021).

The results presented here demonstrated that *Rhizobium leguminosarum* application at all levels of the water regime ameliorates the destructive impacts on growth indices, chlorophylls content and the photosynthetic efficiency of groundnut plants, which might be due to the increase, particularly of macronutrientuptake that could improve the chlorophylls biosynthesis and hence defend the photosynthetic machinery (Abou-Zeid and Abdel–Latif, 2016) also the siderophores′ production which amended the availability of iron (Arora *et al.*, 2001). Moreover, *Bradyrhizobium*-peanut-symbiosis improved plant growth and reduced water stress damage by altering the activities of antioxidant enzymes and increasing osmo-protectants compounds (Barbosa *et al.*, 2018). *Rhizobium*-groundnut-symbiosis in this study showed improvement in the photosynthetic capacity this seemed to concur with the constructive effects on the plant dry weight accumulation. This may be referred to as the availability of the fixed N_2which is a vital element for chlorophylls, and proteins, and essential for the protoplasm formation that initiates cell division and enlargement and eventually increases the growth of the plant. Furthermore, *Rhizobium* inoculation may enhance the uptake of the secreted phytohormones such as gibberellic acid, and indole-3-acetic acid that was convoyed with the abscisic acid decrease, the increase of stomatal opening, CO_2 diffusion, the photosynthetic efficiency (Abou-Zeid *et al.*, 2021) and improve the plant nodulation capacity for nitrogen fixation (Hemon and Sumarjan, 2021). The present finding correlated with our observations that *Rhizobium*-symbiosis leads to increase drought tolerance in faba beans and groundnut (Hussain *et al.*, 2018; Razafintsalama *et al.*, 2022).

N_2-fixation may be affected directly by the activity of nitrogenase or indirectly by leghaemoglobin content, respiratory rate, malate concentration, and nodules structure (Pagare*et al.*, 2019). *Rhizobium* inoculation in the current work showed high N_2 and leghaemoglobin content thereby forming associations of greater symbiotic efficiency as leghemoglobin plays a crucial role in nodules-N_2 fixation which keeps the nodule in a low O_2 environment in addition to facilitating O_2 transport to the bacteroids (Sapna and Sharma, 2021). Plant inoculation with *Rhizobium leguminosarum*-groundnut-symbiosis can allow plants to overcome water deficiency conditions, as D1 and D2 were not limiting factors for the nodule number increase and their fresh and dry weights probably due to the formation of nodules before the application of water stress on the plants. On contrary, Figueiredo *et al.* (2008) postulated that the nodule number, leghemoglobin content, and nitrogenase activity were reduced in common bean plants as a result of drought stress.

It has been concluded that the electrophoretic protein profile of an organism symbolizes its biochemical genetic finger print; each protein band reflects a separated transcriptional episode and affords information concerning the structural genes with their regulatory systems so as to control the biosynthetic pathways of that protein (Li *et al.*, 2015). It is well recognized that abiotic stresses modulate the total amino acid composition and protein metabolism of stressed plants. Besides proline, some free amino acids are concerned with the adaptation of plants through osmotic changes, intonation the permeability of membrane, ion uptake, in addition to gene expression regulation and redox homeostasis (Zemanová *et al.*, 2017). Several genes and transcription factors associated with molecular and physiological changes under drought conditions have been detected (Chen *et al.*, 2012). Under the current study, some new stress protein bands were recognized as a result of water deficits in roots and shoots. The number of bands in roots of D1 and D2

showed 17 and 16 bands and the corresponding numbers for shoots were 15 and 18 bands in comparison with their controls. Clos (1996) reported that dehydrin proteins (MWs of 9–200 KD) which are synthesized in response to drought stress are functional proteins that have a role in stress protection by controlling the formation of structural proteins as well as the proteins which have a role in ease water retention, membrane stability and ions flow (Beck *et al.*,2007).Jha and Subramanian (2018) stated that proteins play important role in the production of energy, structural organization, as well as cell communications, signaling, and division; hence, plants reprogram their assemblage to endure water deficit stress.

Rhizobium inoculation resulted in an increase in the number of bands that reached 16 and 21 bands for D1 and D2-stressed roots and the corresponding number for shoots was 16 bands. This extra number of bands represented new root and shoot different proteins were triggered to withstand this stress and this is similar to the induction of stressed-responsive transcripts (Kawasaki *et al.,* 2001). Where the appearance of these bands may be either due to the merging of a new band or thesplitting of an existing band into its subunits. The newly elaborated protein profile bands that resulted from *Rhizobium* may be also explained on the basis of the mutational event at the regulatory system of an un expectedly activated gene (s) as previously reported by (Hahn *et al.,* 2013). Furthermore, the newly synthesized band with MWs ranges from 15 to 42 KD possibly belongs to small heat shock proteins that might have a role in maintaining the stability of the cell membrane under stress conditions. The disappearance of a few bands reflected the role of drought and/or the *Rhizobium* to reactivate gene(s) that were suppressed (Zhang *et al.,* 2015).

Drought stress and *Rhizobium* incubation lead to improve nitrogen fixation, which in turn leads to the raise of free amino acids and protein synthesis. In accordance with these views treated roots and shoots under water deficiency and *Rhizobium* combination may present genetic effects on the banding pattern. These effects have been manifested as the expression of some novel polypeptides and the disappearance of others (Zemanová *et al.,* 2017). Band intensity varied from one treatment as drought to the other as combined with *Rhizobium*, this would be in turn, traced back to the changes in the expression of the number of structural genes or their regulatory system which would lead to constitutive protein production or lead to attenuation or complete suppression for the concerned genes. Variation in the inoculated and non-inoculated plants under D1 and D2 treatments may declare that *Rhizobium* inoculation modulates the synthesis of protein for harnessing the growth of plants under water stress conditions. Brito *et al.* (2019) findings on peanuts inoculated with *Bradyrhizobium* has the ability to alleviate the harsh effects ofdrought stress via the increase in some geneexpressions that are closely related to the plant signaling and response capacity to stressconditions, suggests akey role in the activation of metabolic cascades for plant protection under water shortage. *Rhizobium-symbiosis* provides osmotic balance; maintains ion homeostasis; and induces drought and salt-responsive genes, metabolic reprogramming, provide transcriptional changes in ion transporter genes (Gupta *et al.,* 2022).

CONCLUSION

Currently, the use of microbes could modulate the plant′s response to adverse environmental conditions. This study pointed out that the drought stress (D1 and D2) was tremendously harmful to the un-inoculated groundnut plants, distinct from the inoculated ones with isolated *Rhizobium leguminosarum* that had notably reduced the damage by increasing the growth biomarkers and photosynthetic pigments and photosynthetic efficiency as

well as changes in protein profile. *Rhizobium* significantly promoted nodulation, leghaemoglobin, and nitrogen content and minimized the deleterious effect of drought in the groundnut plant. Inoculation with *Rhizobium* was found to be highly promising under water stress and can be recommended for further molecular and field-level study.

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