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A Gram-Negative Bacterium, *Sinorhizobium saheli* S-1^{T} Promotes *Vicia faba* Growth Under Irradiance Stress

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ABSTRACT

A local isolate from root nodules of *Acacia nilotica* (L.), nitrogen fixing woody legume tree, grown in the Desert Garden, Aswan University Campus, South-eastern Desert, Aswan, Egypt, was carried out. A purified isolate was designated S-1^{T}, was classified as *Sinorhizobium saheli* according to similarity and phylogenetic analysis of 16S rRNA. S-1^{T} is aerobic Gram-negative rod-shaped strain that was able to fix atmospheric nitrogen to produce NH_3. *S. saheli* S-1^{T} also showed capabilities to produce indole-acetic acid (IAA) and cellulase enzyme. S-1^{T}, as a bio-fertilizer, was able to enhance *Vicia faba* growth under irradiance stress. Pot-culture experiments showed that strain S-1^{T} increased the shoot and root length of *V. faba* by 27.0 and 27.1 cm, respectively, compared to inoculation-free control (negative control). Our findings highlight the role of *S. saheli* S-1^{T} in improving plant growth of *V. faba* under irradiance stress through increasing chlorophyll and proteins content over control treatment and showing maximum photosynthesis rate (*Pn*) at high Photosynthetic Active Radiation levels (*PAR*). It was concluded that *S. saheli* S-1^{T} possessed multiple beneficial effects for *V. faba* productivity when grown under extreme environmental conditions.

Keywords: Biofertilizer; Irradiance stress; Phylogenetic analysis; Photosynthesis rate; Woody legume.

INTRODUCTION

Many physiological processes in plants are affected by irradiance, which is one of the most important environmental factors affecting plant survival, growth, reproduction and distribution (Evans and Poorter, 2001; Muraoka *et al.*, 2002; Keller and Luttge, 2005). In the last few years, ecologists draw a great attention to wild legumes and their symbionts, due to their tolerance to extreme environmental conditions such as severe drought, salinity and elevated temperature. Where, symbiotic rhizobia of naturally growing legumes successfully established good symbiosis (Zaharan, 2001; Khan and Beena, 2002; Abdellah, 2009). *Rhizobia* are Gram negative rods, micro-symbionts and usually known as N_2-fixing bacteria that live as familiar components of soil microbial community, which inhabits the root nodules of most legumes. They are qualified for converting atmospheric nitrogen into ammonia via their nitrogenase complex and then assimilating it into nitrogen containing compounds that promote growth of host plant as previously mentioned (Cleyet-Marel *et al.*, 1990; Gaby and Buckley, 2012). Consequently, association between legume host plants and *Rhizobia* is mutually beneficial. Nitrogen is requiring for all living cells. For a plant it is indispensable element in chlorophyll formation. It also improves plant growth via several mechanisms such as synthesis of amino acids, proteins and hormones and increases plant's resistance to diseases (Singha *et al.*, 2015). Nowadays there is an increasing interest of replacing chemical fertilizers with biofertilizers for its economic, environmental and consumer health reasons (Rigby and Caceres, 2001; Lee and Song, 2007). Yanni *et al.* (2001) stated that *Rhizobium* spp., *Pseudomonas* spp., *Azospirillum* spp. and *Bacillus spp.* are greatly considered as plant growth promoting rhizobacteria (PGPR). The aim of current work is to improve physiological processes of *V. faba* under the influence of irradiance stress by using the recently isolated strain S-1^{T} as Biofertilizer.

MATERIALS AND METHODS

Isolation and phenotypic characterization

Healthy nodules of *Acacia nilotica* (L.), N_2- fixing woody legume tree, grown in the Desert Garden, Aswan University Campus, South Eastern Desert, Aswan, Egypt, were collected. Washed nodules were subjected to sterilization and then sliced, under aseptic condition, in Petri-dish containing mannitol yeast agar (YMA) following the method of Vincent (1970). The plates were incubated at 28± 2^{o}C for 2-3 days. The isolate obtained designated S-1^{T}, was then identified and classified based on phenotypic and biochemical characterizations including colonial morphology, ability to Gram stain, urease, catalase and oxidase production (Christensen, 1946; Kovacs, 1956; Harold, 2002). IAA, NH_3, and cell wall degrading enzymes like amylase and cellulase production were also evaluated (Sarwar *et al*., 1992; Cappuccino and Sherman, 1992).

Genotypic characterization

Genotypic characterization was carried out using 16S rDNA gene amplification (Lane, 1991). The 16S rDNA gene was amplified using the following universal primers, according to White *et al.* (1990), 27F (5’-AGAGTTTGATCCTGGCTCAG-3’) and 1492R (5’-GGTTACCTTGTTACGACTT-3’). The sequence obtained was analyzed using the nucleotide database available at the gene bank, using BLAST tool at National Center for Biotechnology information (NCBI). Phylogenetic trees were constructed using MEGA 7.0 software with neighbor-joining methods.

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Screening of S-1^T for plant growth promoting traits

Infectivity test

The experiment was carried out in the greenhouse, where pots containing 4 kg of sterile sand-clay soil were prepared for planting (1:2). Meanwhile, healthy *Vicia faba* (Giza 843) seeds were sterilized in a 3.5 % NaOCl solution for 5 minutes before being washed completely with sterile distilled water and ready to be planted. Each pot received two sterilized seeds. After seed germination (7-10 days after soaking), pots were randomly divided into three groups (4 pots for each), as follow: control group without any inoculation (negative control), second group was inoculated with KNO_3, as a chemical fertilizer, and considered as reference (positive control). The third group was inoculated with one ml of suspended freshly propagated culture of S-1^T (1×10^9 cells ml^{-1}) and considered a biofertilizer-group following the method of Woomer *et al.* (2011). All pots, designed for the experiment, were irrigated regularly to keep the soil at 9% soil moisture content for 7 weeks.

Plant measurements

Root, shoot lengths and fresh weight

The influence of S-1^T on the growth characteristics of *V. faba* was investigated. Shoot length and fresh weight of plants were measured and recorded as plant growth characteristics. At harvesting time (7 weeks old seedlings), root and shoot lengths were measured using the root intersection method proposed by Newman (1966) and simplified by Tennant (1975). At one day after harvesting, the chlorophyll content of the leaves, as well as soluble and insoluble proteins, was examined (Arnon, 1949; Lowery *et al*., 1951).

Photosynthesis (Pn) and transpiration rate (E)

At the 7th week prior to plants harvesting, photosynthesis (*Pn*) and transpiration rate (*E*) were measured for the plants grown under irradiance stress at photosynthetic active radiation (*PAR*) ranges from 0 to 2500 µmol m^{-2}s^{-1}, that was adjusted by using light module (CI-301LA), based on Research Unit for Study Plants of Arid Lands (RUSPAL)'s Meteorological station readings (Ali *et al.*, 2018). Water use efficiency (*WUE*) was also determined as described by Silva *et al*. (2013) and Ali *et al*. (2018) using the following formula:

$$WUE = \frac{\text{the current net } CO_2 \text{ assimilation rate } (Pn)}{\text{the current transpiration Rate } (E)}$$

Statistical analysis

All the measured parameters were statistically analyzed. Two-way analysis of variance was applied using MINITAB12 statistical software, USA (Minitab Inc, 1998). Data represented are in mean ±SE.

RESULTS

Identification traits of S-1^T

Based on physical traits, pure S-1^T colonies were spherical, milky white, and mucoid. The cells were Gram negative rods. It has the ability to breakdown urea and turns the medium pink. S-1^T can produce oxidase enzyme as well, although catalase recoded as a weak positive. It also has ability to produce IAA hormone.

Molecular identification of S-1^T using 16S rDNA gene (1352 base pair) was revealed that the organism belongs to *Sinorhizobium saheli* strain LMG 7837 (NR026096) with similarity percent 99.70 (Figure 1). The isolate was given the accession number MT341302 in the Gene Bank database.

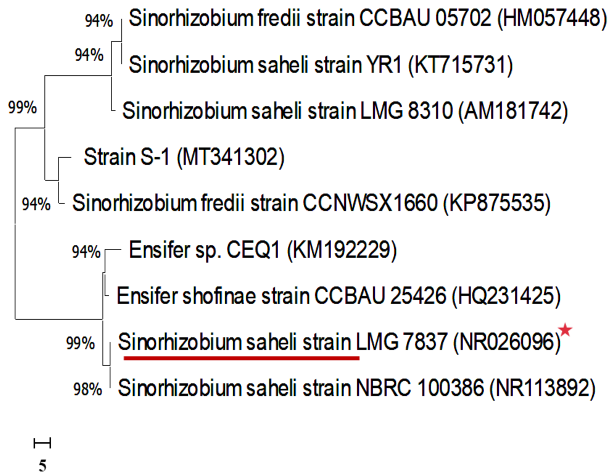

Figure 1: Phylogenetic tree with 100 bootstrap replicates displaying the relationship between *S.saheli* isolated strain (S-1^T) in the present study and the closely related bacteria accessed from NCBI Gen Bank database using MEGA X software

Effect of inoculum on plant growth parameters

Root, shoot lengths and fresh weight

The influence of S-1^T on the growth characteristics of *V. faba* was documented in (Table 1). S-1^T significantly improves ($p \leq 0.001$) the growth of in terms of the longest shoot lengths and roots, as well as the dry weight. The growth parameters of inoculated plants with strain S-1^T, as a biofertilizer, were superior to those of plants fertilized with KNO_3 (positive control) and those left untreated (negative control).

Chlorophyll content and protein content

Similarly, the inoculated plant with S-1^T had significant differences in chlorophyll concentration, soluble and insoluble proteins, and chlorophyll content (Table 1). The chlorophyll content and insoluble protein content of inoculated *Vicia faba* showed a slight significant increase at the $p \leq 0.05$ level. In contrast, when compared to control plants (plant without inoculation), soluble protein concentration increased significantly ($p \leq 0.001$).

Effect of S-1^T on Photosynthesis and transpiration rate under different light levels (Irradiance stress)

A maximum *Pn* of 7.8 µmol m^{-2}s^{-1} was recorded with *V. faba* treated with S-1^T at PAR of 2500 µmolm^{-2}s^{-1} (Fig. 2A). For plant treated with KNO_3 (positive control), a maximum *Pn* of 4.2 µmol m^{-2}s^{-1} was recorded at 1500 µmol m^{-2}s^{-1} *PAR*. Meanwhile, negative values of *Pn* were recorded in case of negative control treatment, i.e. -4.67, -2.41µmol $m^{-2}s^{-1}$, at PAR ranged from 0 to 500 µmol $m^{-2}s^{-1}$ (Fig. 2a). Analysis of variance of *Pn* recorded data in *V. faba* displayed significant variations attributed to differences in both PAR and treatments, where: F=6.67; $p \leq 0.001$, and F=17.31; $p \leq 0.001$, respectively. For positive control (plant treated with KNO_3), a minimum transpiration rate of 1.16 mmol $m^{-2}s^{-1}$ at 9% soil moisture content, was recorded (Fig. 2B) at lowest *PAR* (250µmol $m^{-2}s^{-1}$). On the other hand, *E* in seedlings treated with S-1^T gave a minimum value of 0.62 mmol $m^{-2}s^{-1}$ at *PAR* of 750µmol $m^{-2}s^{-1}$ (Fig. 2B). Data obtained from two-way analysis of variance, showed that *E* significant changes of *V. faba* were attributed to differences in both *PAR* and treatments, where: F=5.24; $p \leq 0.005$, and F=127.27; $p \leq 0.001$, respectively.

Water use efficiency (*WUE*) of *V. faba*

Figure 2C shows the maximum *WUE* value of 9.2 µmol $m^{-2}s^{-1}$ at 2500 µmol $m^{-2}s^{-1}$ of *PAR*. However, positive and negative controls exhibited maximum *WUE* value of 2.9 µmol $m^{-2}s^{-1}$/mmol $m^{-2}s^{-1}$ at 1250 µmol $m^{-2}s^{-1}$ and 3.7 µmol $m^{-2}s^{-1}$/mmol $m^{-2}s^{-1}$at 750 µmol $m^{-2}s^{-1}$ of *PAR*, respectively. From two-way analysis of variance, *WUE* of *V. faba* demonstrated significant changes attributed to differences in both *PAR* and treatments, with F=4.36; $p \leq 0.05$ and F=20.29; $p \leq 0.001$, respectively, using two-way analysis of variance.

DISCUSSION

Irradiance stress is an extreme environmental restriction to agricultural production. The effect of nitrogen-fixing organisms that live in symbiotic relationship with leguminous may play a role to limit this restriction. This study was explored the enhancing effect of local S-1^T rhizobial isolate of *A. nilotica* and the tolerance of *V. faba* growth under irradiance stress. *Rhizobia* play a significant role in conferring resistance and adaptation of plants to irradiance stresses and have potential role in solving future food security issues. An interaction between plant and rhizobia under high irradiance affects not only the plant but also changes soil properties (Williams and Phillips, 1980). Based on analysis of phenotypic and genotypic characters, S-1^T is a *Sinorhizobium saheli* which is widely reported as a beneficial *rhizobium* to colonize *Acacias* (Nick *et al.*, 1999), and was given the name *Sinorhizobium saheli* S-1^T, where S refers to Aswan, the geographic cite of isolation.

Results of the present work showed that our strain, S-1^T, exhibited multiple plant growth promoting traits *in vitro*. Such multiple modes of action have been reported to be the prime reasons of plant's growth promotion and disease suppression ability of plant growth promoting rhizobacteria (Antoun and Prevost, 2005; Bashan and de-Bashan, 2010). We have found that strain S-1^T produces NH_3, cellulase and amylase. In a study done by Sinsabaugh *et al.*, (1991), they have showed that soil enzymes like cellulase, phytase, chitinase, and amylase play decisive role in maintaining soil ecology, fertility and health. Such enhancing growth by *Rhizobia* can be imputed to production of secondary metabolites such as ammonia gas and enzymes that inhibit phytopathogens or by competing for colonization sites, nutrients, etc. (Bhattacharyya and Jha, 2012; Martínez-Viveros *et al.*, 2010).

Furthermore, NH_3 is the preferred nitrogen-containing nutrient for plant growth. Chlorophyll and proteins content are almost proportional to nitrogen content in leaves (Evans, 1983; Singha *et al.*, 2015). Thence, this work showed highest level of chlorophyll content, shoot, root length and fresh weight of plants inoculated with S-1^T compared to other treatments (Table 1), and also nitrogen increases the leaf area of plants, on that way, it influences on photosynthesis (Bojovic and Markovic, 2009).

Table 1: Effect of different treatments on growth parameters, chlorophyll content and soluble and insoluble protein content, of *Vicia faba*. All values are expressed in means ±SD.

Treatment	Growth parameters			Chlorophyll content (mg/g)	Soluble protein content (mg/g)	Insoluble protein content (mg/g)
	Root length (cm $plant^{-1}$)	Shoot length (cm $plant^{-1}$)	Fresh weight (g $plant^{-1}$)			
S-1^T	27.1±0.15	27.0±0.05	25.2±0.30	0.81±0.011	6.10±0.020	3.15±0.040
Positive control	14.0±0.11	25.0±0.11	13.0±0.11	0.77±0.005	3.60±0.002	2.65±0.020
Negative control	9.9±0.11	12.5±0.11	3.0±0.05	0.61±0.010	3.06±0.030	2.40±0.007
Significance	**	**	**	*	**	**

**, high significant ($p \leq 0.001$); *, significant at . the $p \leq 0.05$

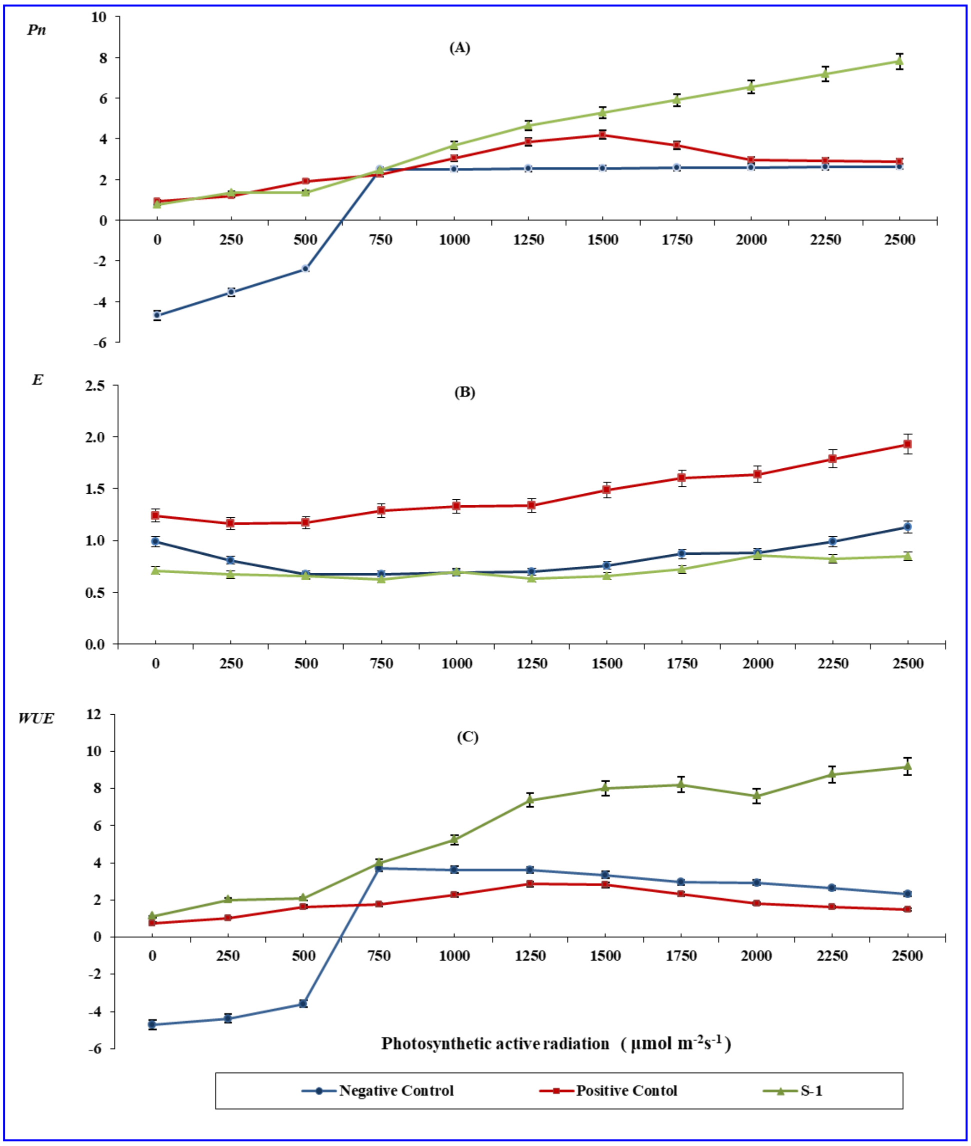

Figure 2: A-C, (A) *Pn* (µmol CO_2 $m^{-2}s^{-1}$); (B) *E* (mmol H_2O m^{-2} s^{-1}), and C, WUE (µmol CO_2 $m^{-2}s^{-1}$/mmol H_2O of *V. faba* under different treatments: Negative control, plants neither inoculated nor fertilized with NH_3; Positive control, Plants fertilized with NH_3; S-1^T, plants inoculated with *Sinorhizobium saheli* S-1^T, at *PAR* ranges from 0 to 2500 µmol $m^{-2}s^{-1}$.

Based on current study's results, maximum value of photosynthesis (Pn) rate in seedling inoculated with S-1^T as PGPR under irradiance stress was recorded. Photosynthesis is a process, which provides the energy necessary for plant growth. Among pigments, chlorophylls, the green pigments, represent the principal pigment responsible for light absorption and photosynthesis (Nelson and Cox, 2004). Moreover, photosynthetic (WUE) is associated with the plant's optimum water use (Robinson *et al.,* 2001; Larcher, 2003; Novriyanti *et al.,* 2012).

As a result of stomatal closure, transpiration rate was minimized, which leads to improvement of water use efficiency (Lawson and Blatt, 2014; Tshikunde *et al.,* 2018). Positive effects of inoculation of common bean with convenient rhizobia strains have been widely reported. Gicharu *et al.* (2013) have registered a significant increase in nodulation when three bean cultivars were inoculated with CIAT 899 (*Rhizobium tropici*) compared to the control treatments. In respect to the inoculation by *Rhizobium*, *Rhizobium* has similar effect on shoot and root dry weights of bean, compared to control, as reported by (Javaid and Mahmood, 2010; Mehrpouyan, 2011; Trabelsi *et al.,* 2011).

CONCLUSION

A. nilotica wild legume inhabiting Aswan region, Egypt was undergone to bacteriological analysis to assay the endophytic bacteria associated with the root nodules of the plant. The potentialities of the isolated bacteria to produce of hydrolases were investigated. It was found that isolated *S. saheli* S-1^T could produce variety of extracellular hydrolytic enzymes including amylase, cellulase and also could produce NH_3 and IAA which play an important role in promoting plant growth. As a supplement to this study, we recommend further studying endophytic bacteria associated with *A. nilotica* that bear extreme environmental conditions to use in agricultural field.

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تحسين نمو نبات الفول ببكتريا *Sinorhizobium saheli* S-1^{T} تحت إجهاد الإشعاع

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الملخص العربــــي

إن العلاقه التكافليه للبقوليات الشجرية مثل شجرة السنط المثبتة للنيتروجين *Acacias nilotica* مع الريزوبيا لها اهمية كبيرة في استصلاح الاراضي القاحلة. قد لفتت أنتباه علماء البيئة خلال السنوات الماضيه بسبب مقدرتها على تحمل الظروف البيئية القاسية. وعلى ذلك ركزت الدراسة الحالية على استخدام عزلات الريزوبيا المعزولة من العقد الجذرية لل *Acacia nilotica* L كأسمدة بيولوجية لنباتات الفول المعرضه للاجهاد الإشعاعي بدلاً من استخدام الأسمدة الكيميائيه التي تؤدى إلى مشاكل بيئية وتلوث المياة الجوفية ومصادر المياة الاخري. واهتمت الدراسة بتعريف البكتريا المعزولة ، وفقا للتحليل الجيني لعينة البكتيريا على أنه *Sinorhizobium saheli* S-1^{T}. و أوضحت النتائج أن الكائن المعزول له القدرة على إنتاج كل من NH_3 و إنزيمات التحلل المائى كالاميليز وهرمون أندول حمض الخليك كما اثبتت الدراسه إن حقن نبات الفول ب S-1^{T} مقارنة ب KNO_3 كسماد كميائي، أدت الى تعزيز نمو النبات كما ادى الى تحسين العمليات الفسيولوجية كالبناء الضوئى والنتح تحت مدى قيم اشعاع (0-2500ميكرومول م$^{-2}$ ث$^{-1}$) و التى تم قياسها وتقيمها باستخدام جهاز التحليل الغازى بالاشعه تحت الحمراء.