Bradyrhizobium japonicum Strain Asw1 Colonizing Cowpea (Vigna unguiculata L.) Roots Mediates Eco-physiological and Growth Responses in Faba Bean (Vicia faba L.) and Wheat (Triticum aestivum L.)

Noura Sh.A. Hagaggi, Usama A.A. Radwan
Botany Department, Faculty of Science, Aswan University, Aswan 81528, Egypt.

INTRODUCTION

In the twenty-first century, the greatest challenge in the agriculture is the increment of the crop productivity with reducing the harmful implications resulting from the utilization of chemical fertilization. Plant-microbe interaction provide an essential function for plant growth promotion and crop productivity with safety management and controlling ecosystems pollution. In this research, Bradyrhizobium japonicum strain Asw1 (accession no. MN079045) isolated from Vigna unguiculata (L.) nodules were used to mediate some important eco-physiological responsiveness included the photosynthetic rate (Pₖ), transpiration rate (Tₑ), stomatal conductance (Gₑ) and leaf water-use efficiency (WUE) in faba bean and wheat plants. The results obtained indicated that the inoculation with strain Asw1 was significantly (P < 0.05) enhanced the eco-physiological and growth responses in both plant species. Our research provides a considerable sign for the benefit use of Bradyrhizobium japonicum Asw1as plant growth promoting symbiont. Therefore, we suggest the exploitation of strain Asw1 in large scale, in the agricultural purposes.

Keywords: Bradyrhizobium japonicum, Faba bean, Photosynthesis, Stomatal conductance, Transpiration, Wheat.

Introduction

Recently, the main challenge of agriculture is the crop production expanding. For this purpose, chemical fertilizers and pesticides are extensively used. With the passage of time, these practices cause huge risk to environment and health (Piranooshe et al., 2011). Therefore, attempting to use eco-friendly biological fertilizers as an alternative to chemical fertilizers will achieve good resonance; including reduce costs, improve soil fertility and protect the environment from pollution risks (Piranooshe et al., 2011).

Faba bean (Vicia faba L.) and wheat (Triticum aestivum L.) are species of flowering plants that related to family Fabaceae and Poaceae respectively. They are among the most important crops that widely cultivated in most countries worldwide for humans and animals’ consumption as sources of calories, protein and carbohydrates (Matthews & Marcellos, 2003; Zaki et al., 2007). In Egypt, faba bean and wheat represent the main crops for human diet. They provide most of the total daily protein consumption and calorie intake as well as they have significant strategic roles due to their contribution in Egyptian income (Gabrial, 1982; Rowntree, 1993; Abdel Ghaffar, 1994).

Rhizobacteria had proven their efficiency long time ago as plant growth promoting and they successfully used for this purpose (Bhattacharjee et al., 2009; Lugtenberg & Kamilova, 2009). They benefit the plants in different ways such as biological nitrogen fixation, phytohormones and siderophore production, phosphate solubilization and enhance plant defense to pathogens (Antoun & Prevost, 2005; Mehboob et al., 2008; Bhattacharyya & Jha, 2012). So far, family Rhizobiaceae comprises six genera: Rhizobium, Mesorhizobium, Sinorhizobium, Azorhizobium, Bradyrhizobium and Allorhizobium. Bradyrhizobium japonicum is a

*Corresponding author email: nourasharkawi@sci.aswu.edu.eg  Tel.: 01156536624
Received 3/1/2020; Accepted 28/6/2020
DOI : 10.21608/eqbo.2020.21904.1428
Edited by: Prof. Dr. Fawzy M. Salama, Faculty of Science, Assuit University, Assuit, Egypt.
©2020 National Information and Documentation Center (NIDOC)
species of Gram-negative symbiotic rhizobacteria that has an effective role as plant growth-promoting via biological nitrogen fixation, production of phytohormones and siderophores (Werner, 1992). This bacterium lives symbiotically with leguminous plant forming nodules along their root system and also capable of colonizing the roots of non-legumes (Antoun et al., 1998).

This study offers valuable findings that *Bradyrhizobium japonicum* Asw1 significantly mediated the eco-physiological and growth responses in faba bean and wheat plants.

**Materials and Methods**

All experiments were performed in the Research Unit for Study Plants of Arid Lands (RUSPAL), Biotechnology and Eco-physiology Labs, Aswan University, Egypt.

**Sampling, isolation and identification**

Pink colored nodules were collected from cowpea (*Vigna unguiculata* L. Walp) roots cultivated in RUSPAL Greenhouse. The root nodules were placed in sterile plastic bags and were immediately carried to the laboratory for study.

Surface sterilization of nodules was performed using 70% ethanol and 5% sodium hypochlorite and then washed five times with sterilized distilled water (Vincent, 1970). Nodules were crushed by sterilized forceps in a sterile test tube containing one ml of sterile saline solution. Loopful of the nodule suspension was streaked on the surface of yeast extract mannitol agar (YMA) plates (In g/L: Mannitol 10, K$_2$HPO$_4$ 0.5, MgSO$_4$ 7H$_2$O 0.2, NaCl 0.1 and yeast extract 0.5). Medium was supplemented with Congo red (10ml/L of a stock Congo red 1/400 (w/v) aqueous solution) to differentiate between rhizobial isolates and contamination (Vincent, 1970). Plates were incubated at 28±2°C for 72hrs for the appearance of colonies. The colonies obtained were picked up and purified by repeated streaking on fresh prepared YMA plates, and the purified cultures were maintained on slants at the refrigerator for further experimentation.

The isolate was identified based on morphological and biochemical characteristics as well as 16S rRNA gene sequencing. Morphological and biochemical characteristics were determined according to Bergey’s Manual of Determinative Bacteriology (Bergey, 2009). 16S rRNA gene sequencing was performed commercially in Macrogen, Inc., Korea. The obtained sequence was analyzed using the National Center for Biotechnology Information (NCBI) databases (https://www.ncbi.nlm.nih.gov/) and was deposited into NCBI databases to get an accession number. The evolutionary history was inferred by the Neighbor-Joining method (Saitou & Nei, 1987) and the evolutionary distances were computed by the Maximum Composite Likelihood method (Tamura et al., 2004) using MEGA X software (Kumar et al., 2018).

**Nitrogen fixation ability**

Nitrogen fixation ability of the isolate was qualitatively examined followed the method of Dobereiner & Day (1975). Isolate was streaked on the surface of nitrogen free malate agar plates supplemented with bromothymol blue as an indicator. Plates were incubated for 72hrs at 28±2°C. Change in color of the medium from pale green to blue indicated positive result. This related to the increase in pH due to the formation of ammonia and nitrates from the atmospheric N$_2$ fixation.

**Phosphate solubilization**

Phosphate solubilizing ability of the isolate was qualitatively tested by streaking on Pikovskaya’s (PVK) agar medium (Pikovskaya, 1948). PVK plates were incubated for 72hrs at 28±2°C and were daily observed for the formation of clear halo zones.

**Indol acetic acid (IAA) production**

IAA production by the present isolate was estimated using Salkowski’s reagent (Ehmann, 1977). Isolate was grown in nutrient broth supplemented with 0.5% L-tryptophan and incubated at 28±2°C under 150 rpm for 72hrs. Culture was centrifuged at 5000rpm for 15min. A mixture of 1ml of the obtained supernatant and 1ml of Salkowski’s reagent (2.0ml of 0.5M FeCl$_3$ in 98.0ml of 35% HClO$_4$) was kept for 30min in the dark at room temperature. Absorbance of developed pink color was measured at 535nm. IAA concentration was calculated in µg/ml using IAA standard curve.

**Preparation and inoculation technique**

Sterilized yeast extract mannitol broth (150ml) was inoculated with 1ml of the present strain suspension (1×10$^7$ CFU/ml). Culture was incubated at 28±2°C for 72hrs with shaking at 150rpm (Vincent, 1970). Cell concentration approximately 10$^9$ CFU/ml was prepared using McFarland standard (McFarland, 1907).

Faba bean and wheat seeds were obtained from...
Faculty of Agriculture and Natural Resources, Aswan University. They were surfacesteerilized with 70% ethanol for 3 min, and then washed three times with sterile distilled water.

For each plant species, sterilized seeds were inoculated with strain Asw1 by soaking them for 2 hrs in 100 mL inoculum suspension (1 × 10⁷ CFU/ml) and 4% carboxy methylcellulose solution as an adhesive agent to achieve uniform coverage. Seeds soaked in sterile distilled water were used as the control. Seeds were air dried before sowing.

**Pot experiment**
Twenty plastic pots packed with autoclaved mixture of clay and sand (2:1, v/v) were used for sowing. Water regime was maintained at 90% field capacity using soil moister meter (model 5910A) (Sheded & Radwan, 2008). Pots were kept under controlled conditions in the growth chamber at 30±1°C, photosynthetically active radiation (PAR) of 400-700 μmol m⁻²s⁻¹ and 12: 12 hrs of light: darkness.

After eight weeks, six homogenous plants of each species were chosen for eco-physiological measurements. Photosynthesis rate (Pᵣ), transpiration rate (Tᵣ), and stomatal conductance (Gₑ) of the youngest healthy fully expanded leaves were measured using the portable photosynthesis system (CI-340) and light module (CI-301LA) (CID Bio-Science, Inc., WA, USA). Measurements were carried out in controlled leaf chamber at relative humidity of 50%, temperature of 26±0.1°C, CO₂ level of 360 ppm and PAR ranged from 0 to 2500 μmol m⁻²s⁻¹. Leaf water-use efficiency (WUE) in (μmol CO₂·μmol⁻¹ H₂O) was calculated according to the following formula (Kirkham, 2005):

\[
WUE = \frac{Pᵣ}{Tᵣ}
\]

where: (Pᵣ) is the current net CO₂ assimilation rate; and (Tᵣ) is the current transpiration rate.

**Growth parameters**
Plant height (cm), number of leaves per plant, fresh and dry weight of shoot (g/plant) of random samples from each control and inoculated plants were evaluated.

**Estimation of leaf total nitrogen content**
Total nitrogen of the leaves of both control and inoculated plants was estimated according to the method of Kjeldahl (1883). 1 g of dry leaves was digested with 10 ml of conc. sulphuric acid and 5 g of catalyst mixture (K₂SO₄; CuSO₄; Se; 50:10:1). After digestion, 10 mL of distilled water was added. The distillate was collected in 250 mL capacity conical flask containing 20 ml of 4% boric acid solution and 4 drops of methyl red indicator. 40 mL of 40% NaOH was added. The distillate mixture was titrated with 0.02 N sulphuric acid until the appearance of pinkish color. The following formula was used to determine the total nitrogen content percent.

Leaf total nitrogen content (\%) = ((Sample titer-blank titer) X Normality of acid X Atomic weight of nitrogen X 100)/ (Sample weight (g) X 1000)

**Determination of total carbohydrates**
Total carbohydrates were quantified by an anthrone method according to Morris (1948). Absorbance was read at 620 nm using UV-Visible Spectrophotometer, Model UVmini-1240 (Shimadzu Corporation, Japan).

**Determination of total proteins**
Total proteins were determined following the method described by Lowry et al. (1951).

**Statistical analysis**
Significant differences of all the obtained data were determined using one-way analysis of variance (ANOVA) from MINITAB statistical program version 12 (Minitab Inc., 1998). Values were represented by the means ± standard deviations and P<0.05 was considered as significant.

**Results**
In this study, rhizobacterial strain Asw1 was isolated from *Vigna unguiculata* (L.) root nodules. Morphological and biochemical characteristics of the strain were investigated and recorded in Table 1.

The present strain was identified based on its morphological and biochemical characters in addition to its 16S rRNA gene sequence. The obtained sequence was blasted with the NCBI reference sequence database. Results revealed high similarity (99.93%) to *Bradyrhizobium japonicum* strain 311b6 (accession no. NR036865) and it was deposited in NCBI databases under the accession number MN079045. Phylogenetic relationships of strain Asw1 and closely related bacteria derived from NCBI Genbank based on 16S rRNA gene sequence were represented in the form of rectangular phylogram using Neighbor-joining method in MEGA X software (Fig. 1).

Strain Asw1 was able to solubilize calcium phosphate and exhibited phosphate-solubilizing

*Egypt. J. Bot.* **Vol. 60**, No.3 (2020)
zone of 7mm diameter. Furthermore, it could fix atmospheric N\textsubscript{2} and produce significant amount of IAA (20.58μg/ml).

### TABLE 1. Morphological and biochemical characteristics of strain Asw1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony morphology</td>
<td>Circular, opaque and convex</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>White</td>
</tr>
<tr>
<td>Cells</td>
<td>Rods (0.5-1.0 x 1.5-3.0μm)</td>
</tr>
<tr>
<td>Ability to Gram staining</td>
<td>-</td>
</tr>
<tr>
<td>Spore formation</td>
<td>-</td>
</tr>
<tr>
<td>Starch hydrolysis</td>
<td>+</td>
</tr>
<tr>
<td>Urease test</td>
<td>+</td>
</tr>
<tr>
<td>Indole formation</td>
<td>+</td>
</tr>
<tr>
<td>H2S production</td>
<td>+</td>
</tr>
<tr>
<td>Voges-Proskauer</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase test</td>
<td>+</td>
</tr>
<tr>
<td>Citrate utilization</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>-</td>
</tr>
<tr>
<td>Fermentation of:</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>-</td>
</tr>
</tbody>
</table>

+: Positive; -: Negative

After eight weeks from sowing, plants were subjected to some eco-physiological measurements included photosynthesis rate (P\textsubscript{r}), transpiration rate (T\textsubscript{r}), stomatal conductance (G\textsubscript{s}) and water use efficiency (WUE). The obtained results showed that B. japonicum Asw1-inoculated faba bean plants revealed higher efficiency of the photosynthetic rate in comparison with the non-inoculated plants under high light level (Fig. 2 a).

Interestingly, it was observed that the maximum photosynthesis rate (13.370μmolm\textsuperscript{-2}s\textsuperscript{-1}) of the inoculated faba bean plants that was recorded at PAR level of 2500μmolm\textsuperscript{-2}s\textsuperscript{-1} was significantly higher than the maximum photosynthesis rate (3.522μmolm\textsuperscript{-2}s\textsuperscript{-1}) of the control plants that was noticed at PAR level of 2250μmolm\textsuperscript{-2}s\textsuperscript{-1} (Fig. 2 a). Consequently, the inoculation with the present strain was significantly enhanced the photosynthesis rate (F=6.12; P< 0.05). On the other hand, photosynthesis rates in both control and inoculated wheat plants were recorded negative values of -0.20 and -0.69μmol m\textsuperscript{-2} s\textsuperscript{-1} respectively at zero μmol m\textsuperscript{-2} s\textsuperscript{-1}PAR level. With increasing the PAR levels, the inoculated wheat plants showed higher photosynthesis rates than the control plants. At PAR level of 500μmol m\textsuperscript{-2} s\textsuperscript{-1}, the photosynthesis rate increased to 1.12μmol m\textsuperscript{-2} s\textsuperscript{-1} and reached its maximum rate (6.26μmolm\textsuperscript{-2}s\textsuperscript{-1}) at PAR level of 2500μmol m\textsuperscript{-2} s\textsuperscript{-1} (Fig. 3 a). Statistical analysis of data indicated that the photosynthesis rate of wheat plants significantly changed due to the inoculation (F=7.24; P< 0.05).

---

**Fig. 1.** Phylogenetic relationships of strain Asw1 and other closely related species based on 16S rRNA gene sequences by Neighbor-joining method using MEGA X software.

Fig. 2 a-d. Changes of photosynthesis rate (a), transpiration rate (b), stomatal conductance (c) and leaf water-use efficiency (d) in *Vicia faba* mediated by strain Asw1 [Values are mean ± standard errors (SEs) of five independent biological replicates (n = 5)].

Fig. 3 a-d. Changes of photosynthesis rate (a), transpiration rate (b), stomatal conductance (c) and leaf water-use efficiency (d) in *Triticum aestivum* mediated by strain [Values are mean ± standard errors (SEs) of five independent biological replicates (n = 5)].
The maximum transpiration rate (0.94 mmol m⁻² s⁻¹) in the control faba bean plants was recorded at zero μmol m⁻² s⁻¹ PAR. Other wise, the transpiration rate of the inoculated plants was ranged from 0.15 to 0.62 mmol m⁻² s⁻¹ at different levels of PAR (Fig. 2 b). It was observed that the inoculation has a profound impact on the transpiration rate of faba bean plants (F=102.03; P<0.0001). Further, the transpiration rates in both the inoculated as well as the control wheat plants reached their maximum values at PAR level of 2500 μmol m⁻² s⁻¹. The inoculated plants showed higher transpiration rate than the control plants which were 3.32 mmol m⁻² s⁻¹ and 1.82 mmol m⁻² s⁻¹, respectively (Fig. 3 b). Significantly, the inoculation mediated the transpiration rate changes in wheat plants (F=12.57; P<0.005).

The maximum stomatal conductance of the inoculated faba bean plants was 345.29 μmol m⁻² s⁻¹ recorded at low PAR (750 μmol m⁻² s⁻¹), where as it was 71.54 μmol m⁻² s⁻¹ in the control plants observed at higher PAR level of 2500 μmol m⁻² s⁻¹ (Fig. 2 c). From data analysis, the stomatal conductance in faba bean plants showed significant changes attributed to the inoculation (F=49.37; P<0.0001). Other wise, in the control wheat plants, the highest value of stomatal conductance (162.7 μmol m⁻² s⁻¹) was recorded at zero μmol m⁻² s⁻¹ PAR. However, the stomatal conductance in the inoculated plants reached the triple value (645.57 μmol m⁻² s⁻¹) in comparison with the control plants at PAR level of 2500μmol m⁻² s⁻¹ (Fig. 3 c). It was found that the inoculation was significantly affected stomatal conductance in wheat plants (F=18.21; P<0.0001).

Results indicated that faba bean plants inoculated with strain Asw1 was significantly enhanced the leaf wateruse efficiency (F= 40.149; P<0.00001). It was observed that the leaf wateruse efficiencyof the inoculated plants were six times higher than that of the control plants (Fig. 2 d). Otherwise, data analysis revealed that the inoculation had no significant effect on water use efficiency in wheat plants (F= 2.87; P=0.1053) (Fig. 3 d).

Growth parameters for both faba bean and wheat plants attribute to strain Asw1 inoculation in comparison with the control plants were recorded in Table 2. They revealed that the heights, number of leaves, fresh and dry weights of both faba bean and wheat plants were significantly increased due to the inoculation with strain Asw1 compared with control plants. The best nitrogen content was markedly noted in leaves of the inoculated plants of both species. In both plant species, significant increases (P< 0.05) in the total carbohydrates and total proteins contents were detected in inoculated plant relative to control.

**Discussion**

To accomplish the highest crops productivity in agriculture, chemical fertilizers which are expensive and risky to the environment and the health must be used. Subsequently, the use of eco-friendly biofertilizers as an alternative was effective (Rigby & Caceres, 2001).

Numerous Plant Growth Promoting Rhizobacteria (PGPR) such as species of *Rhizobium, Mesorhizobium, Bradyrhizobium, Azorhizobium, Allorhizobium, Sinorhizobium, Pseudomonas and Bacillus* are recognized for their capacity to stimulate plant growth and improve soil fertility and crop productivity through many natural processes including nitrogen fixation, phosphate solubilization, phytohormones production and increasing the plant defense against diseases (Werner, 1992; Trinick & Hadobas, 1995; Yanni et al., 1997, 2001; Biswas, 1998; Rigby & Caceres, 2001; Peng et al., 2002; Matiru & Dakora, 2004).

**TABLE 2. Growth parameters of inoculated *Vicia faba* and *Triticum aestivum* plant vs. control plants.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th><em>Vicia faba</em></th>
<th><em>Triticum aestivum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Inoculated</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>43.1±1.2</td>
<td>56.8±1.6</td>
</tr>
<tr>
<td>Number of leaves/plant</td>
<td>25±2</td>
<td>48±1</td>
</tr>
<tr>
<td>Fresh weight of shoot (g)/plant</td>
<td>59.6±1.5</td>
<td>73±3.5</td>
</tr>
<tr>
<td>Dry weight of shoot (g)/plant</td>
<td>8.3±2.1</td>
<td>10.3±1.7</td>
</tr>
<tr>
<td>Leaf total nitrogen content (%)</td>
<td>3.8±0.4</td>
<td>4.26±0.3</td>
</tr>
<tr>
<td>Total proteins (mg g⁻¹ DW)</td>
<td>44.5±0.4</td>
<td>57.8±0.4</td>
</tr>
<tr>
<td>Total carbohydrates (mg g⁻¹ DW)</td>
<td>36.2±0.3</td>
<td>52.6±2.3</td>
</tr>
</tbody>
</table>

*Values are mean ± standard errors (SEs) of five independent biological replicates (n=5).*

*Egypt. J. Bot. Vol. 60, No.3 (2020)*
In the current research, the interesting ability of *Bradyrhizobium japonicum* strain Asw1 to fix atmospheric nitrogen, solubilize phosphate, and to produce indole acetic acid involving in the plant growth promotion make our strain an effective selection to use as a biofertilizer. Furthermore, this strain associates in abundance with the root nodules of cowpea plant, and therefore it can be used directly at no cost by cultivating the land with cowpea before sowing faba bean and wheat crops.

To overcome feeding problem resulting from the continuous increasing population, crops productivity improvements of an urgent requirement (Kromdijk & Long, 2016). In Egypt, it is known that faba bean and wheat possess great importance as sources of carbohydrates and proteins for human and animal diet. Therefore, these plants were chosen for this study to improve their growth and productivity via improving their eco-physiological traits.

The obtained results showed that *B. japonicum* Asw1-inoculated faba bean plants revealed higher efficiency of the photosynthetic rate in comparison with the non-inoculated plants under high light level (Fig. 2 a). In wheat, at zero μmol m⁻² s⁻¹ PAR, negative values of photosynthetic rate were recorded in both inoculated and control plants (Fig. 3 a). The negative photosynthetic values resulted from the dark respiration by the plants for producing energy during growth (Jones, 2014). In parallel, inoculation of wheat plants interestingly promoted photosynthesis rate at high PAR level of 2500μ molm⁻² s⁻¹ relative to the control (Fig. 3 a). Early study by Imsande (1989) stated the increment of photosynthetic rates of soybean plants inoculated with *Bradyrhizobium japonicum*. Photosynthetic rate of Alaska pea (*Pisum sativum* L.) also increased following rhizobial inoculation (DeJong & Phillips, 1981). It is worth mentioning that with increasing photosynthesis rates, the biomass production and crop yields also increase (Tollenaar, 1991; Makino, 2011).

Water loss by plants is essential for photosynthesis process. Plants get water from the soil and CO₂ from the air via their roots and their stomata respectively. There is a gradient in vapor pressure between the dry air and the wet mesophyll of the leaf, this makes water to be pulled from the soil to the leaves and comes it out to the air through the stomata (Bierhuizen & Slatyer, 1965; Stuart et al., 1985; Taiz & Zeiger, 1998). In the present research, it was observed that the transpiration rate in inoculated faba bean plants at higher light levels were slow (Fig. 2 b, c). Interestingly, it was found that the inoculation with strain Asw1 prevented the excess water loss and save water content of the leaves relative to the control. Thus, the inoculation supported cellenlargement via maintain turgor (Raven et al., 1992).

Crop development, growth and production are greatly affected by water limitation (Faroq et al., 2009). In the current research, transpiration rates recorded by strain Asw1-inoculated wheat plants were higher than that of the control (Fig. 3 b). Consequently, strain Asw1 could be efficiently used to enhance water uptake by the plants through improving the transpiration rate. Therefore, plant growth subsequently promotes.

Plant stomata serve as gates for the two important plant processes, photosyn thesis and transpiration. Opening and closing of stomata regulate CO₂ uptake and water evaporation in response to environmental factors. By increasing stomatal conductance, gas exchange (CO₂ and water) regulate. Thus, CO₂ uptakes by plants grow under well-watered conditions will increase and subsequently promote photosynthesis (Willmer & Fricker, 1996). In the present research, the inoculation of both faba bean and wheat plants with strain Asw1 enhanced stomatal conductance (gas exchange) which in turn promoted the photosynthetic rates (Fig. 2 c and 3 c). Previously, it was stated that rhizobial inoculation of rice plant improved the stomatal conductance and as a result increased the photosynthetic rates by 12% (Peng et al., 2002).

Investigation to find potential mechanisms for enhancing water use efficiency (WUE) became urgent to improve crop yield and water management. Plant WUE can regulate either genetically, by environmental factors or by cultivation methods (Zhang & Shan, 2002). Increasing WUE of plants leads to decreasing stomatal conductance and maintains high photosynthetic rates (Farquhar & Sharkey, 1982; Wang et al., 2010). In the current research, it was found that WUE infaba bean plants was significantly improved due to the inoculation, while that of wheat plants was not considerably affected (Fig. 2 d and 3 d), respectively.

We assumed that the inoculation of faba bean and wheat plants with strain Asw1 which resulted...
in a significant changes in the eco-physiological traits may subsequently effect on plant growth. For this purpose, we performed some important estimates to indicate whether growth improved or not. These estimates included growth parameters, leaf total nitrogen content, total carbohydrates and total proteins. Data recorded for both inoculated and control plants was statistically analyzed and accurately compared to determine the efficiency of strain Asw1 to be used as a biofertilizer for promoting plant growth. Remarkably, inoculated faba bean and wheat plants exhibited heightened, number of leaves, fresh and dry weights of shoots, leaf total nitrogen, total carbohydrates and total proteins contents much higher than those of control plants (Tables 2 and 3). Enhancement of growth responses attributed to Bradyrhizobium japonicum inoculation was previously reported for soybean plants (Masciarelli et al., 2014). This provided us a good indication for the possibility of using strain Asw1 as a plant growth promoting rhizobacterium.

**Conclusion**

A rhizobacterial strain Asw1 was isolated from cowpea (Vigna unguiculata L.) nodules and identified using morphological, biochemical and molecular characterization. It revealed a close genetic relationship to Bradyrhizobium japonicum. The strain Asw1 exhibited significant ability to fix atmospheric nitrogen, solubilize calcium phosphate and produce indole acetic acid phytohormone. This encouraged us to highlight this strain for using it as a biofertilizer to promote plant growth. Benefit eco-physiological effects attribute to the inoculation with strain Asw1 were evaluated in both faba bean and wheat plants. Interestingly it was observed that the photosynthetic rate (P) transpiration rates ($T_s$) as well as stomatal conductance ($G_s$) were significantly enhanced in the inoculated plants of both species compared with the non-inoculated control plants (P< 0.05). Consequently, growth parameters, leaf total nitrogen, total carbohydrates and total proteins contents were noticeably enhanced in both plant species matched with the control. Therefore, we recommend focusing on strain Asw1 as a biofertilizer in agriculture, which will achieve a significant improvement in plant growth and crop productivity.

**Acknowledgement:** Many thanks to the Research Unit for Study Plants of Arid Lands and Botany Department, Faculty of Science, Aswan University for their supporting and providing the requirements of scientific research.

**Conflict of interests:** The authors declare that they have no conflict of interest.

**Authors contribution:** Both authors conceived, designed research, conducted experiments and wrote the manuscript. Noura Sh. A. Hagaggi contributed new analytical tools and Usama A.A. Radwan analyzed data. Both authors read and approved the manuscript.

**Ethical approval:** Not applicable.

**Funding:** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**References**


Kirkham, M.B. (2005) "Water-use Efficiency". Kansas State University Manhattan, KS, USA, Elsevier Ltd.


McFarland, J. (1907) Nephelometer: an instrument for media used for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *Journal of the American Medical Association*, 14, 1176-8.


Agriculture and Ecology, 2, 492-501.


In the twenty-first century, the biggest challenge in agriculture is increasing crop productivity with reduced negative effects from chemical fertilizers. Microbial interactions play a crucial role in enhancing plant growth and crop productivity by improving crop yield. In this study, strain Asw1 of Bradyrhizobium japonicum (isolated from cowpea roots) was used to evaluate some important physiological and environmental responses, including photosynthetic efficiency, nitrogen fixation, water use efficiency, and growth responses in cowpea and wheat. The results showed that infection by strain Asw1 was statistically significant (P<0.05) compared to non-infected plants. This enhanced the environmental and physiological responses and plant growth in both plant species. Our study indicates the benefits of using strain Asw1 as a growth enhancer in agriculture. We therefore propose that strain Asw1 be used in agricultural practices.