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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.)

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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-microbeinteraction provide an essential function for plant growth promotion andcrop productivity with safety management and controlling ecosystems pollution. In this research, *Bradyrhizobiumjaponicum* strain Asw1 (accession no. MN079045) isolated from *Vignaunguiculata* (L.) nodules were used to mediate some important eco-physiological responsiveness includedthe photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (G_s) and leaf water-use efficiency (WUE) in faba bean and wheat plants. The results obtained indicated that the inoculation with strain Asw1was 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 *Bradyrhizobiumjaponicum* Asw1as plant growth promoting symbiotant. Therefore, we suggestthe 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 andhealth (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 agoas 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

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species of Gram-negative symbiotic rhizobacteria that has aneffective 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 infaba 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 contained one ml of sterile saline solution. Loopful of the nodule suspension was streaked on the surface of yeastextractmannitol agar (YMA) plates (In g/L: Mannitol 10, K_2HPO_4 0.5, $MgSO_4$. $7H_2O$ 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^{o}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).

Nitrogenfixation 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^{o}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^{o}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^{o}C under 150 rpm for 72hrs. Culturewas 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 strainsuspension (1×10^7 CFU/ml). Culture was incubated at 28±2^{o}C for 72hrs with shaking at 150rpm (Vincent, 1970). Cell concentration approximately 10^7 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. Theywere surfacesterilized with 70% ethanol for 3min, and then washed three times with sterile distilled water.

For each plant species, sterilized seeds were inoculated with strain Asw1 by soaking them for 2hrs in 100mL inoculum suspension (1×10^7 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 400-700µmol $m^{-2}s^{1}$ and 12: 12hrs of light: darkness.

After eight weeks, six homogenous plants of each species were chosen for eco-physiological measurements. Photosynthesis rate (P_n), transpiration rate (T_r), and stomatal conductance (G_s) 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, CO_2 level of 360ppm and *PAR* ranged from 0 to 2500µmol $m^{-2}s^{-1}$.Leaf water-use efficiency (WUE) in (µmol CO_2µmol^{-1} H_2O) was calculated according to the following formula (Kirkham, 2005):

$$WUE = \frac{Pn}{Tr}$$

where: (P_n) is the current net CO_2 assimilation rate; and (T_r) 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 leaftotal nitrogen content

Total nitrogen of the leaves of both control and inoculated plants was estimated according to the method of Kjeldahl (1883). 1g of dry leaves was digested with 10 ml of conc. sulphuric acid and 5g of catalyst mixture (K_2SO_4: $CuSO_4$: Se; 50:10:1). After digestion, 10mL of distilled water was added. The distillate was collected in 250mL capacity conical flask containing 20mL of 4% boric acid solution and 4 drops of methyl red indicator. 40mL of 40% NaOH was added. The distillate mixture was titrated with 0.02N 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 quantifiedby an anthrone method according to Morris (1948). Absorbance was read at 620nm 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 3I1b6 (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 zone of 7mm diameter. Furthermore, it could fix atmospheric N_2 and produce significant amount of IAA (20.58µg/ml).

TABLE 1. Morphological and biochemical characteristics of strain Asw1.

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

+: Positive; -: Negative

After eight weeks from sowing, plants were subjected to some eco-physiological measurements included photosynthesis rate (*P_n*), transpiration rate (*T_r*), stomatal conductance (*G_s*) and water use efficiency (*WUE*). The obtained results showed that *B. japonicum* Asw1-inoculated faba bean plants revealed higher effciency 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^{-2}s^{-1}) of the inoculated faba bean plants that was recorded at *PAR* level of 2500µmolm^{-2}s^{-1} was significantly higher than the maximum photosynthesis rate (3.522µmolm^{-2}s^{-1}) of the control plants that was noticed at *PAR* level of 2250µmolm^{-2}s^{-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^{-2} s^{-1} respectively at zero µmol m^{-2} s^{-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^{-2} s^{-1}, the photosynthesis rate increased to 1.12µmol m^{-2} s^{-1} and reached its maximum rate (6.26µmolm^{-2}s^{-1}) at *PAR* level of 2500µmol m^{-2} s^{-1} (Fig. 3 a). Statistical analysis of data indicated that the photosynthesis rate of wheat plants significantly changed due to the inoculation ($F=7.24$; $P< 0.05$).

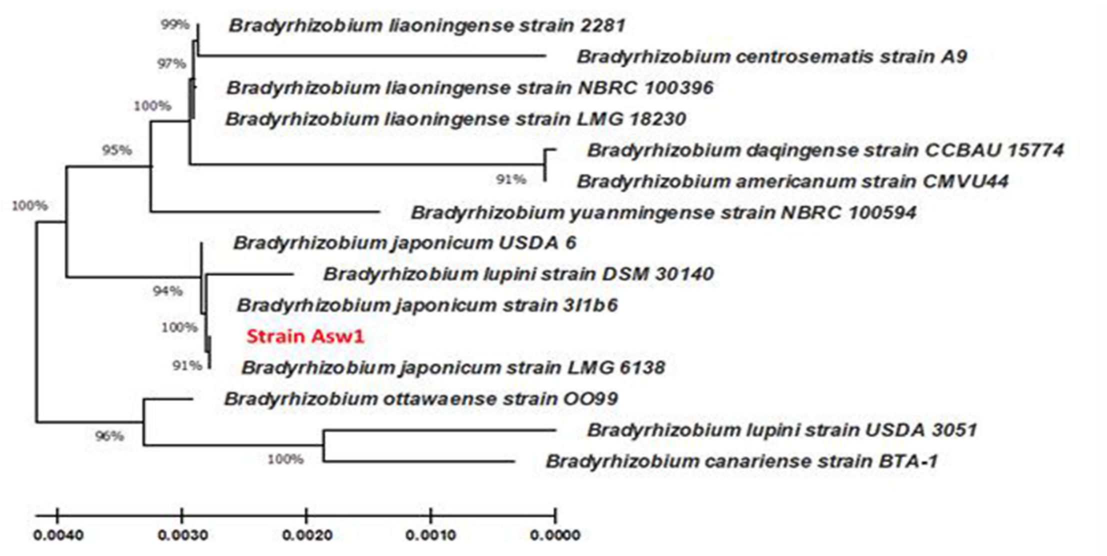

Fig. 1. Phylogenetic relationships of strain Asw1 and other closely related species based on 16S rRNA gene sequences by Neighbor-joining method using MEGA X software.

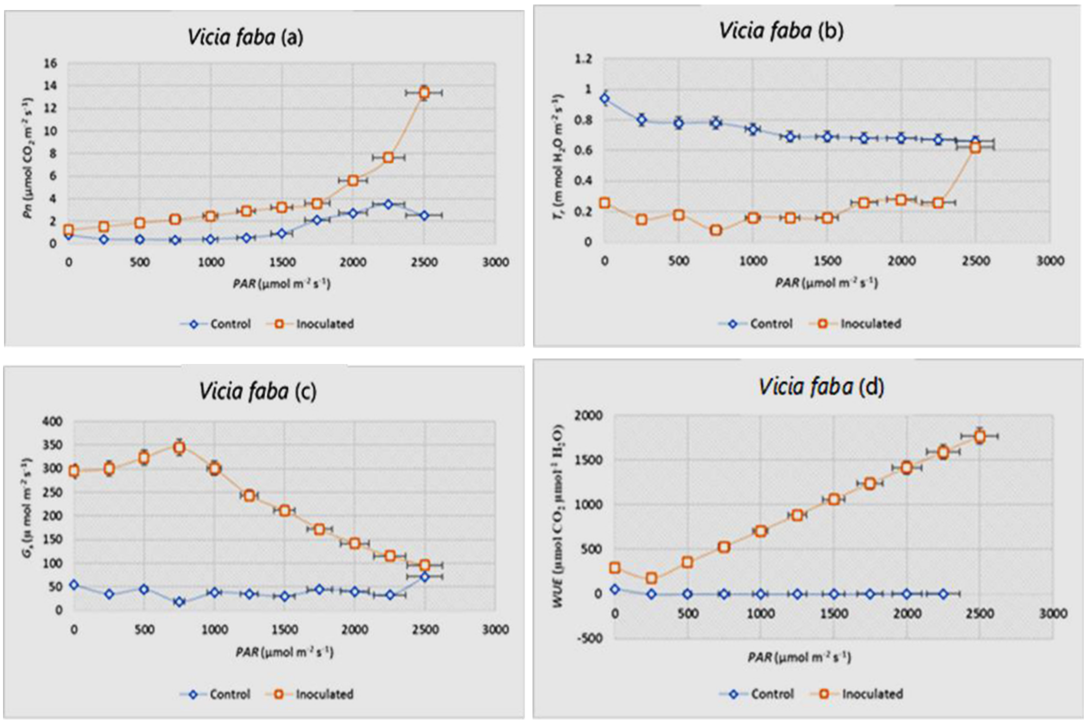

Fig. 2 a-d. Changes of photosynthesis rate (a), transpiration rate (b), stomatal conductance (c) and leaf water-use efficiency (d) in *Vicia faba* mediated by strain Asw1 [Values are mean ± standard errors (SEs) of five independent biological replicates (n =5)].

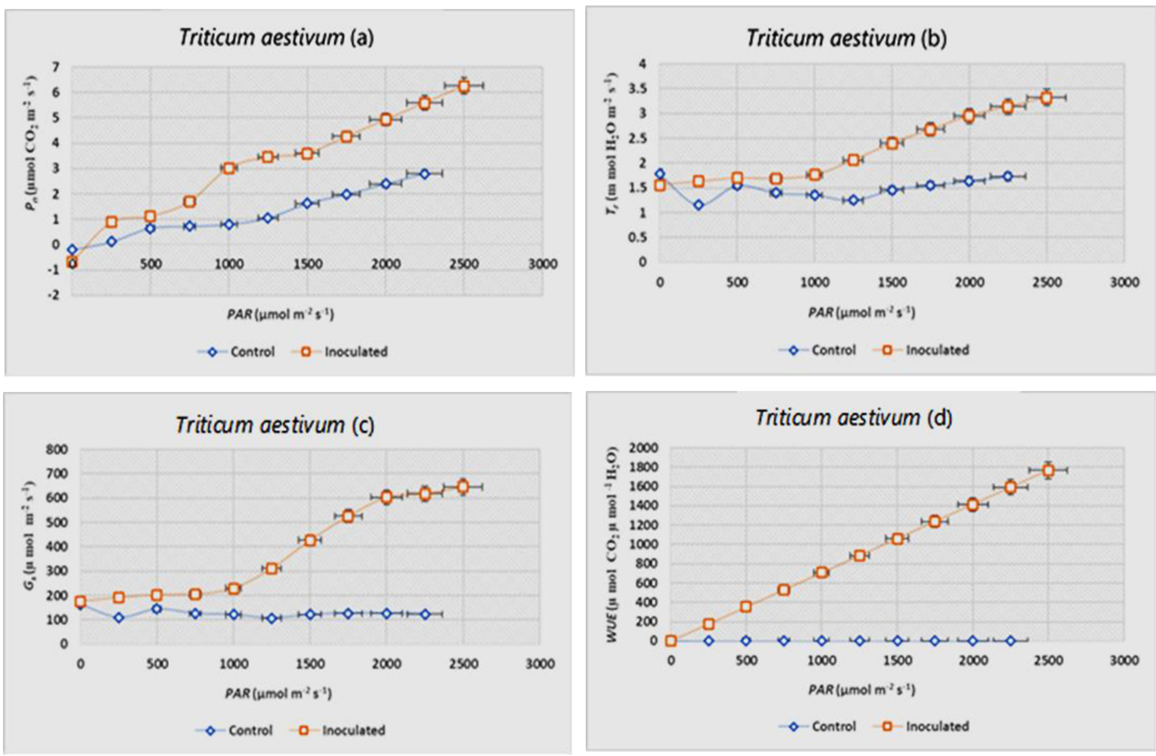

Fig. 3 a-d. Changes of photosynthesis rate (a), transpiration rate (b), stomatal conductance (c) and leaf water-use efficiency (d) in *Triticum aestivum* mediated by strain [Values are mean ± standard errors (SEs) of five independent biological replicates (n =5)].

The maxim umtranspiration rate (0.94mmolm^{-2}s^{-1}) in the control faba bean plants was recorded at zero µmolm $^{-2}$s^{-1}*PAR*. Other wise, the transpiration rateof the inoculated plants was ranged from 0.15 to 0.62 mmolm^{-2}s^{-1} at different levels of *PAR* (Fig. 2 b). It was observed that the inoculation has a profound impact on the transpirationrate 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 345.29µmolm^{-2}s^{-1} recorded at low *PAR* (750µ molm^{-2}s^{-1}), where as it was 71.54µmolm^{-2}s^{-1} in the control plants observed at higher *PAR* level of 2500µ molm^{-2}s^{-1} (Fig. 2 c). From data analysis, the stomatal conductance in fababean 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 (162.7µmol 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 (645.57µmol 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 srelative 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 *Viciafaba* and *Triticum aestivum* plant svs. control plants.

	Vicia faba		*Triticum aestivum*	
Parameters	**Control**	**Inoculated**	**Control**	**Inoculated**
Height (cm)	43.1±1.2	56.8±1.6	59.7±1.4	83.1±0.5
Number of leaves/plant	25±2	48±1	20±1	26±3
Fresh weight of shoot (g)/plant	59.6±1.5	73±3.5	43.3±0.2	50.7±1.5
Dry weight of shoot (g)/ plant	8.3±2.1	10.3±1.7	5.2±0.7	8.4±1.2
Leaf total nitrogen content (%)	3.8±0.4	4.26±0.3	2.5±0.5	5.9±1.7
Total proteins (mg g^{-1} DW)	44.5±0.4	57.8±0.4	31.3±1	40.1±1.5
Total carbohydrates (mg g^{-1} DW)	36.2±0.3	52.6±2.3	34.2±1.1	45.3±1.5

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

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 improvementis 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-inoculatedfaba bean plants revealed higher effciency 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 mol$m^{-2}s^{-1}$ 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 CO_2 from the air via their roots and their stomata respectively. There is a gradient in vapor pressure between the dry air andthe 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 inoculatedfaba 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 cellenlargementvia maintain turgor (Raven et al., 1992).

Crop development, growth and production are greatly affected by water limitation (Farooq 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 Asw1could 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 CO_2 uptake and water evaporation in response to environmental factors. By increasing stomatal conductance, gas exchange (CO_2 and water) regulate. Thus, CO_2 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 Asw1enhanced 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 urgentto 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 heights, 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 Asw1as 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_n), transpiration rates (T_r) 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 enhancedin both plant species matched with the control. Therefore, we recommendfocusing on strain Asw1 as a biofertilizer in agriculture, which will achieve a significant improvement in plant growth and crop productivity.

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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.

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References

Abdel Ghaffar, A. (1994) The producing, pricing and marketing policies of wheat in the Government of Egypt. Cairo. Mimeo.

Antoun, H., Prevost, D. (Eds.) (2005) Ecology of plant growth promoting rhizobacteria. In: "*PGPR: Biocontrol and Biofertilization*". Springer, Dordrecht, pp. 1-38.

Antoun. H., Beauchamp, Ch. J., Goussard, N., Chabot, R., Lalande, R. (1998) Potential of Rhizobium and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: Effect on radishes (*Raphanussativus* L.).*Plant and Soil,* **204**, 57-67.

Bergey, D.H. (2009) "*Bergey's Manual of Systematic Bacteriology*". 2nd ed. Springer.

Bhattacharjee, R.B., Singh, A., Mukhopadhyay, S.N. (2009) Use of nitrogen-fixing bacteria as biofertilizer for non-legumes: prospects and challenges. *Applied Microbiology and Biotechnology,* **80**, 199-209.

Bhattacharyya, P.N., Jha, D.K. (2012) Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World Journal of Microbiology and Biotechnology,* **28**, 1327-1350.

Bierhuizen, J.F., Slatyer, R.O. (1965) Effect of atmospheric concentration of water vapour and CO_2 in determining transpiration-photosynthesis relationships of cotton leaves. *Agricultural Meteorology,* **2**, 259-270.

Biswas, J.C. (1998) Effect of nitrogen fixing bacteria on growth promotion of lowland rice (*Oryza sativa* L.). *Ph.D. Thesis*. Philippines University, Los Banos˜.

DeJong, T.M., Phillips, D.A. (1981) Nitrogen stress and apparent photosynthesis in symbiotically grown *Pisumsativum* L. *Plant Physiology,* **68**, 309-313.

Dobereiner, J., Day, J.M. (1975) Associative symbiosis in tropical grasses: Characterization of microorganisms and dinitrogen fixing sites. Proceedings of the 1st International Symposium on Nitrogen Fixation, (SNF' 75), Washington State University Press, Pullman, pp. 518-538.

Ehmann, A. (1977) The Van Urk-Salkowski reagent-a sensitive and specific chromogenic reagent for silica gel thin-layer chromatographic detection and identification of indole derivatives. *Journal of Chromatography,* **132**, 267-276.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A. (2009) Plant drought stress: Effects, mechanisms and management. *Agronomy for Sustainable Development,* **29**, 185-212.

Farquhar, G.D., Sharkey, T.D. (1982) Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology,* **33**, 317-345.

Gabrial, G.N. (1982) The role of faba beans in the Egyptian diet. In: "*Faba Bean Improvement*", Hawtin, G., Webb, C. (Eds.), pp. 309-317, The Netherlands: MartinusNijhoff Publishers.

Imsande, J. (1989) Rapid dinitrogen fixation during soybean pod fill enhances net photosynthetic output and seed yield: a new perspective. *Agronomy Journal,* **81**, 549-556.

Jones, H.G. (2014) "*Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology*". 3rd ed. Cambridge University Press, New York, USA.

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

Kjeldahl, J. (1883) A New Method for the Determination of Nitrogen in Organic Matter. *Zeitschriftfür Analytische Chemie,* **22**, 366-382.

Kromdijk, J., Long, S.P. (2016) One crop breeding cycle from starvation? How engineering crop photosynthesis for rising CO_2 and temperature could be one important route to alleviation. *Proceedings of the Royal Society B: Biological Sciences,* **283**.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution,* **35**, 1547-1549.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951) Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265-275.

Lugtenberg, B., Kamilova, F. (2009) Plant-Growth-Promoting Rhizobacteria. *Annual Review of Microbiology,* **63**, 541-556.

Makino, A. (2011) Photosynthesis, grain yield, and nitrogen utilization in rice and wheat. *Plant Physiology,* **155**, 125-129.

Masciarelli, O., Llanes, A., Luna, V. (2014) A new PGPR co-inoculated with *Bradyrhizobium japonicum* enhances soybean nodulation. *Microbiological Research,* **169**, 609-615.

Matiru, V.N., Dakora, F.D. (2004) Potential use of Rhizobial bacteria as promoters of plant growth for increased yield in landraces of African cereal crops. *African Journal of Biotechnology,* **3**(1), 1-7.

Matthews, P., Marcellos, H. (2003) "*Faba Bean*", 2nd ed. Agfact P4.2.7. Division of Plant Industries, NSW, Australia.

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.

Mehboob, I., Zahir, Z.A., Mahboob, A., Shahzad, S.M., Jawad, A., Arshad, M. (2008) Preliminary screening of Rhizobium isolates for improving growth of maize seedlings under axenic conditions. *Soil and Environment,* **27**, 64-71.

Minitab Inc. (1998) Users' Guide 2: Data Analysis and Quality Tools, Release 12:12, Minitab Inc.

Morris, D.L. (1948) Quantitative determination of carbohydrates with Dreywood'santhrone reagent. *Science,* **107**, 254-255.

Peng, S., Biswas, J.C., Ladha, J.C., Gyaneshwar, P., Chen, Y. (2002) Influence of rhizobial inoculation on photosynthesis and grain yield of rice. *Agronomy Journal,* **94**, 925-929.

Pikovskaya, R.I. (1948) Mobilization of phosphorus in soil connection with the vital activity of some microbial species. *Microbiology,* **17**, 362-370.

Piranooshe, H., Emam, I., Jamali, R. (2011) Comparison of bio-fertilizers with chemical fertilizers on growth, yield and oil content of sunflower (*Helianthus nnuss* L.) at different levels of drought stress. *Journal of*

Agriculture and Ecology, **2**, 492- 501.

Raven, P.H., Evert, R.F., Eichhorn, S.E. (1992) "*Biology of Plants*". Peter H. Raven, Ray F. Evert, Susan E. Eichhorn (Eds.). New York: New York: Worth.

Rigby, D., Caceres, D. (2001) Organic farming and the sustainability of agricultural systems. *Agricultural Systems,* **68**, 21-40.

Rigby, D., Caceres, D. (2001) Organic farming and the sustainability of agricultural systems. *Agricultural Systems,* **68**, 21-40.

Rowntree, J. (1993) Marketing channels and price determination for agricultural commodities. In: "*The Agriculture of Egypt*", E.M. Craig (Ed.). Oxford, U.K., Oxford University Press.

Saitou, N., Nei, M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution,* **4**, 406-425.

Sheded, M.G., Radwan, U.A.A. (2008) Plant water relations as an indicator of drought tolerance of *Senna* speciesin the Egyptian Nubia. *World Applied Sciences Journal,* **3**(3), 382-390.

Stuart, B.L., Krieg, D.R., Abernathy, J.R. (1985) Photosynthesis and Stomatal-conductance responses of Johnsongrass (*Sorghum halepense*) to Water Stress. *Weed Science,* **33**(5), 635-639.

Taiz, L., Zeiger, E. (1998) "*Plant Physiology*". U.S.A.: Sinauer Associates, Inc.

Tamura, K., Nei, M., Kumar, S. (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* (USA), **101**, 11030-11035.

Tollenaar, M. (1991) Physiological-basis of genetic improvement of maize hybrids in Ontario from 1959 to 1988. *Crop Science,* **31**, 119-124.

Trinick, M.J., Hadobas, P.A. (1995) Formation of nodular structures on the non-legumes *Brassica napus*, *B.campestris*, *B. juncea* and *Arabidopsis thaliana* with *Bradyrhizobium* and *Rhizobium* isolated from *Parasponia* spp. or legumes grown in tropical soils. *Plant Soil,* **172**, 207-219.

Vincent, J.M. (1970) "*A Manual for the Practical Study of the Root-nodule Bacteria*". IBP15. Blackwell Scientific Publications, Oxford and Edinburgh. U.K.

Wang, Y., Liu, F., Andersen, M.N., Jensen, C.R. (2010) Improved plant nitrogen nutrition contributes to higher water use efficiency in tomatoes under alternate partial root-zone irrigation. *Functional Plant Biology,* **37**, 175-182.

Werner, D. (1992) “*Symbiosis of Plants and Microbes*”. Chapman & Hall, London.

Willmer, C.M., Fricker, M.D. (1996) “*Stomata*”. 2nd ed. London: Chapman & Hall.

Yanni, Y.G., Rizk, R.Y., Corich, V., Squartini, A., Ninke, K., Philip-Hollingsworth, S, Orgambide, G., de Bruijn, F., Stoltzfus, J., Buckley, D., Schmidt, T.M., Mateos, P.F., Ladha, J.K., Dazzo, F.B. (1997) Natural endophytic association between *Rhizobium leguminosarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. *Plant Soil,* **194**, 99-114.

Yanni, Y., Rizk, R., Abd-El Fattah, F. (2001) The beneficial plant growth-promoting association of *Rhizobium leguminosarum*bv. *trifolii* with rice roots. *Australian Journal of Plant Physiology*, **28**, 845-870.

Zaki, E., Nabila, M., Hassanein, M.S., Gamal El-Din, K. (2007) Growth and yield of some wheat cultivars irrigated with saline water in newly cultivated land as affected by biofertilization. *Journal of Applied Sciences Research,* **3**(10), 1121-1126.

Zhang, S.Q. and Shan, L. (2002) The research advance of water use efficiency in plants. *Agricultural Research in the Arid Areas,* **20**, 1-5.

بريديريزوبيوم جابونيكوم سلالة Asw1 المستعمِرة لجذور اللوبيا تتوسط الاستجابات الفسيولوجية البيئية واستجابات النمو في الفول والقمح

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في القرن الحادي والعشرين، التحدي الأكبر في الزراعة هو زيادة إنتاجية المحاصيل مع تقليل الآثار الضارة الناتجة عن استخدام التسميد الكيميائي. يوفر التفاعل بين الميكروبات النباتية وظيفة أساسية لتعزيز نمو النبات وإنتاجية المحاصيل مع إدارة السلامة والتحكم في تلوث النظم البيئية. في هذا البحث، تم استخدام *Bradyrhizobium japonicum* سلالة Asw1 (540970NM) المعزولة من العقد الجذرية لنبات اللوبيا لتتوسط بعض الاستجابات الفسيولوجية البيئية المهمة بما في ذلك معدل التمثيل الضوئي، معدل النتح، التوصيل الثغري وكفاءة استخدام المياه الورقية في نباتات الفول والقمح. أشارت النتائج التي تم الحصول عليها إلى أن التلقيح بالسلالةAsw1 كان معنويا (P<0.05) يعزز الاستجابة البيئية والفسيولوجية واستجابات النمو في كلا النوعين النباتيين. يقدم بحثنا علامة كبيرة على فائدة استخدام *Bradyrhizobium japonicum* سلالة Asw1 كمتكافل معزز لنمو النبات. لذلك نقترح استغلال السلالةAsw1 على نطاق واسع في الأغراض الزراعية.