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Growth and Photosynthetic Characteristics of Sesame Seedlings with Gibberellin-Producing *Rhodobacter sphaeroides* SIR03 and Biochar

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Abstract: The use of plant growth-promoting rhizobacteria (PGPR) with biochar is apprised to be a promising bio-fertilizer for improving the soil fertility and plant growth and development. The current study aimed to identify a potential plant growth-promoting rhizobacterium alongside biochar to improve sesame seedling productivity. Our results revealed that among the nine isolates, SIR01, SIR03, and SIR07 significantly improved the growth and biomass of sesame and Waito-C rice seedlings. The increase in growth of Waito-C rice seedlings through isolate SIR01, SIR03, and SIR07, suggests their ability to produce phytohormones such as GA4, GA9, GA24, and GA34. Furthermore, the application of isolate SIR03 and biochar together revealed a synergistic increase in sesame seedling growth and biomass (fresh and dry weight) compared with their individual applications. This may be explained by enhancement of photosynthetic rate, chlorophyll fluorescence, stomatal conductance, and transpiration rate by the combined SIR03 and biochar treatment. This suggests that co-inoculation with SIR03 alongside the application of biochar can be considered an eco-friendly, low-cost bio-fertilizer to potentially improve sesame seedling growth and development.

Keywords: PGPR; biochar; GA production; sesame seedling

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1. Introduction

Climate change has exacerbated the impact of abiotic stressors on crops, reducing crop development and yield, and consequently, limiting global agricultural production [1]. Continued industrialization and greenhouse gas emissions are anticipated to increase global land surface temperatures by 5–9 °C by the end of the century [2]. Only 3.5 percent of the world's territory is spared from the effects of climate change [1]. Plant growth and physiology are harmed by such climatic variations, which cause many abiotic stresses and, as a result, limit their yield. Climate change harms the Earth's crust, causing irregular and erratic precipitation, rising temperatures, and the establishment of drought-prone zones [3]. Therefore, sustainable farming methods have been acknowledged as cost-effective means of achieving food security [4]. However, the management of chemical fertilizer is one of the most difficult aspects of a transition to sustainability [5]. Biochar manufactured from a variety of raw materials such as straw, livestock manure, and municipal solid waste (sewage sludge) has been widely used in horticulture and agriculture [6], and prepared through the process of pyrolysis or dry carbonization, which involves burning the material at high temperatures while operating under anaerobic conditions [7,8]. Because of its affordability and benefits for food security, waste biomass is now widely employed to produce biochar. The direct mechanism of biochar is the increased availability of crucial nutrients in the soil, including K+, and the decreased absorption of Na+ [9]. Biochar can boost soil organic matter content, enhance drainage and permeability, improve water and nutrient retention, boost cation exchange capacity (CEC), and affect the microclimate [10]. Depending on the feedstock, pyrolysis, and composting conditions, biochar can contain

significant macro- and micronutrients for plants. Of all the macronutrients, nitrogen is the most crucial for plant growth and is also the most heat-sensitive. A sizeable amount of nitrogen (between 0.04 and 2.4%) is present in biochar [11], but because this nitrogen is heterocyclic, only a limited proportion of it can be released into soils. By reducing ammonia volatilization, nitrous oxide emission, and nitrogen leaching, biochar can lower compost and soil nitrogen losses [12]. Biochar can further affect plant growth and microbial community structure by exerting direct effects on soil quality [13]. Sludge biochar has been recommended as a helpful soil supplement because it improves soil attributes, such as structure, infiltration rates, water holding capacity, soil respiration, pH, fertility, and plant development [14,15]. Biochar can also improve the availability of trace elements to plants and soil microbes, reduce the bioavailability of potentially harmful elements in soils, and inhibit the uptake and transport of these pollutants by plants [16]. Furthermore, sludge biochar has the potential to increase soil carbon pools while lowering greenhouse gas emissions such as CH_4 and N_2O [17,18]. The unique features of biochar, such as its large internal surface area and capacity to sorb soluble organic materials, gases, and inorganic nutrients, have been shown to cause complicated interactions between biochar and soil components [19]. These interactions are likely to also occur when biochar is utilized as a compost supplement [20]. Compost microorganisms colonize the surface of biochar, due to the increased surface area available to the microorganisms, favorable moisture levels resulting from the increased water-holding capacity, improved micro-aeration, and sorption of available carbon compounds that the microorganisms can readily use [21].

Moreover, plant growth-promoting rhizobacteria (PGPR) can increase crop development under abiotic stress through various direct and indirect processes [22]. Plants inoculated with stress-tolerant PGPR are protected from the negative effects of stress and exhibit less plant growth inhibition. Bacterial exopolysaccharides (EPS) promote nutrient absorption and indirectly alter N_2 fixation and phosphate solubilization in non-legumes by binding free phosphorus [23]. Similarly, the potential of photosynthetic bacteria (PSB) has been largely overlooked. A phylogenetically diverse group, PSB, is well known for its wide distribution in water and its metabolic versatility, which includes nitrogen and carbon dioxide fixation and desulfurization [24]. Photosynthetic bacteria have been extensively used in agricultural production to promote plant growth and improve crop quality [25]. These bacteria can thrive in anaerobic or aerobic environments and can fix N_2 and CO_2 using either organic or inorganic materials as electron donors [25,26]. They can be found in a variety of natural settings, including wetlands, salt marshes, lakes, lagoons, wastewater, ponds, sediment, moist soils, and wetland ecosystems. [27,28]. Similarly, with the ACC deaminase activity, production of exopolysaccharides that bind Na+ and reduce its uptake in plants, up- or down-regulation of stress-responsive genes, and accumulation of osmolytes, PGPR can enhance crop growth in water deficit and salt-affected soils by alleviating the negative effects of stress-induced ethylene [29]. When combined with organic amendments, PGPR has the potential to improve not only crop yield, but also the physicochemical and biological features of the soil [30]. The PGPR in the soil acts as a biochemist that can influence soil pH, contribute to plant biomass, increase enzyme activity, and improve mineralization (C and N) processes. Plant growth-promoting rhizobacteria and biochar are two new biostimulants that have been shown to increase metabolism and confer tolerance in crops that have been exposed to a variety of biotic and abiotic challenges [31]. Biochar and PGPR are physiologically safe technologies that boost plant metabolism through a symbiotic relationship. The generation of secondary metabolites, ion control, improved ion absorption through roots, and translocation to shoots are all functional components of PGPR [32]. Biochar, however, replenishes the soil, improves carbon sequestration, and promotes microbial development [33]. Numerous studies have shown PGPR treatment to be advantageous for plant growth and development. Abideen (2020) [34] found that biochar promoted the development of *Phragmites karka* under water stress, and Kammann (2011) [35] found that adequate doses of biochar improved the growth of *Chenopodium quinoa* under water stress. Furthermore, PGPR and biochar are well-known to enhance the growth of a variety of

leguminous crops [36]. Plant growth-promoting properties such as abscisic acid (ABA) regulation [37], gibberellin (GA) synthesis [38], and phosphate, potassium, and silicate solubilization [39] have all been linked to PGPMs, and biochar has been shown to aid PGPM growth, prevent illness, regulate heavy metal concentrations, boost soil fertility, and alleviate severe biotic and abiotic stresses [40].

Sesame is one of the important industrial crops and its oil is rich in nutrition. Sesame seeds have been used as a healthy dietary source for disease prevention in Asian nations for many thousands of years. According to a paper, sesame includes chemicals with antioxidant, anticancer, antiaging, cholesterol-lowering, antihypertensive, and antimutagenic activities [41]. Linoleic, palmitic, and stearic acids, which together make up roughly 96 percent of all fatty acids, are found in the highest concentrations in sesame oil [42]. Sesame seeds have a high oil content, a delectably nutty scent, a moderate flavor, and are suitable for making biodiesel. [43]. In addition to being a good source of vitamin B and E, sesame seeds are also a significant source of carbohydrates, proteins, fiber, important minerals (Ca, P, Fe, etc.), tryptophan, methionine, lignans, flavonoids, phenolics, saponins, and polyunsaturated fatty acids [44]. Sesame seed consumption was USD 6559.0 million globally in 2018, and it is predicted that this amount will increase to USD 7244.9 million by 2024 [45]. Food and oilseed crops should be produced continuously to satisfy consumer demand, which is rising. Thus, producers utilize non-renewable inorganic fertilizers excessively, which occasionally causes a problem in the soil and prevents the interaction between roots and soil microbes in the rhizosphere [46]. Therefore, a better approach is to choose a biologically active, sustainable, and friendly method.

There are minimal data on the synergistic use of PGPR and biochar, and little specifically on the effects of PGPR and biochar on sesame plant productivity. As a result, the current study was designed to evaluate the synergistic use of biochar and photosynthetic bacteria with GA-producing ability to stimulate growth and improve respiration and photosynthesis rates in sesame seedlings. The findings of this study are expected to aid in the development of novel management strategies for increasing growth and output, while also boosting soil fertility. Furthermore, the interaction of PGPR with biochar might be used as a strategy to replenish soil nutrient resources in the future, resulting in a more sustainable ecosystem.

2. Materials and Methods

2.1. Collection and Isolation of Photosynthetic Bacteria from Soil

To identify and screen for photosynthetic bacteria, we collected 14 paddy soil samples from rice fields from different regions of Daegu, including Namwon (35.401515, 127.366626), Geoje (35.000728, 128.695338), Pohang (36.018564, 129.307004), Gimcheon (36.105075, 128.123664), and Samcheok (37.389239, 129.215630). The soil samples were enclosed in individual sterilized zip bags and stored in an ice box at 0–6 °C for safe transportation to the plant physiology laboratory. Then, 1g of each soil sample was first serially diluted (10-1 to 10-9) using 0.85% saline water. Bacterial strains were isolated by plating 300 µL of each serial dilution on LB agar media and incubating at 28 °C until the formation of bacterial colonies. The obtained bacterial colonies were further purified by streaking on LB agar media until pure and clean colonies appeared, and they were then incubated for 36 h at 28 °C. The obtained purified colonies were evaluated for morphological characteristics, such as size, color, shape, and growth pattern, for the identification and differentiation of bacterial isolates. Furthermore, 20 random isolates were selected for further screening and their growth promoting activities. These isolates were grown on LB medium containing sodium succinate as an organic carbon source, at 28 °C and 3000 lux light intensity under aerobic conditions for 48 h; among these isolates, nine isolates—SIR01, SIR02, SIR03, SIR04, SIR05, SIR06, SIR07, SIR08, and SIR09—were further selected based on their colony morphology, growth, mobility, red color, and their phytohormonal (GAs) production ability.

2.2. Screening Bioassay for Growth Promotion in Sesame Seeds

To investigate the seed germination and growth potential of the bacterial isolate, nine strains were tested on sesame seeds. The sesame seeds were surface-sterilized with 2.5% sodium hypochlorite for 5 min. The sterilized seeds were placed on autoclave filter paper in a petri dish (10 seeds/Petri dish), and 5 mL of each bacterial strain was inoculated into seeds and kept for germination under controlled conditions (14/10 h light/dark, 28/24 °C, 70% relative humidity, 250 mol m^{-2} s^{-1} light intensity) for 5 days. Five days after germination, the fresh weight and length of the sesame seeds were measured.

2.3. Identification and Phylogenetic Analysis of the Isolated Endophytic Bacteria

After screening for different traits and growth promotion on sesame seeds, the most effective bacterial isolate, SIR03, was identified by PCR amplification and sequencing of 16S ribosomal RNA (rRNA) genes using the 27F primer (5′- AGAGTTTGATC (AC) TGGCTCAG-3′) and 1492R primer (5′-CGG (CT) TACCTTGTTACGACTT-3′). NCBI BLAST and EzTaxon were used to determine the homology of different nucleotide sequences of the selected isolate, while MEGA 6.1 software was used for phylogenetic analysis. Our results revealed that isolate SIR03 exhibited high sequence identity with *Rhodobacter sphaeroides* NCBI GenBank accession number MW345829.

2.4. Quantification of Gibberellins in the Culture Broth

For measurement of gibberellins, selected strains of isolate SIR03 were cultivated in nutrient broth (120 mL) for 7 days at 30 °C (in a shaking incubator at 120 rpm), and were then centrifuged (10,000× *g*) and filtered (0.45 m filter paper). The separated culture filtrate (CF) was subjected to GC-MSSIM and examined for GAs. A modified approach by Lee et al. (2019) [47] was used to determine the amount of GAs. Several internal GAs standards (17-2H2) were used (obtained from Prof. Lewis N. Mander, Australian National University, Canberra, Australia). All extracts for distinct fractions were run on a C18 column (90–130 mm; Waters Corp., Milford, MA, USA). For GC–MS, the injection volume of all aliquots was fixed at 1 mL for each type of GA. The peak area ratios were used to calculate the amount of bioactive GAs (GA1, GA3, GA4, and GA7) and inactive GAs (GA8, GA9, GA12, GA19, GA20, GA24, and GA36) in the CF. A hydrocarbon standard was also used to determine retention times.

2.5. Screening Bioassay for Plant Growth Promotion in Waito-C Rice

Experiments on the mutant rice cultivar Waito-C (GA-deficient) were carried out to investigate the growth-stimulating capacity and gibberellin production. In a shaking incubator, sterilized Waito-C rice seeds were inoculated with selected bacterial isolates (109 cfu/mL) for 6 h, whereas the control seeds were treated with autoclave distal water under the same conditions. The inoculated seeds were grown for 7 d in 0.8 percent agar-media in a controlled environment (14/10 h light/dark, 28/24 °C, 70% relative humidity, 250 mol m^{-2} s^{-1} light intensity), and 7 days after sowing, plant growth characteristics were recorded.

2.6. Experiment Location, Method, and Design

The experiment was conducted in a greenhouse at Kyungpook National University, Daegu. The *R. sphaeroides* SIR03 was incubated for 5 days at 30 °C in a shaking incubator at 200 rpm in a broth medium. The bacterial suspension (cells) was diluted with sterile distilled water to a final concentration of 108 CFU/mL. Sesame seeds were purchased from Greenheart Bio Co. (Daegu, Korea), surface sterilized with sodium hypochlorite (5%) for 10 min, and thoroughly rinsed with autoclaved double-distilled water (DDW). Seeds were sown in plastic trays containing horticultural soil and grown under controlled greenhouse conditions (30 ± 2 °C). The composition of the horticultural soil was as follows: peat moss (13–18%), perlite (7–11%), coco-peat (63–68%), and zeolite (6–8%), with macronutrients being NH_{4+}~90 mg/kg, $NO_3{}^-$~205 mg/kg, P_2O_5~350 mg/kg, and K_2O~100 mg/kg (auto-

claved three times). Two-week-old sesame seedlings (50 per treatment) were transplanted to pots and treated with 5 mL of bacterial culture and a mixture of horticultural soil and 1% biochar. After two weeks, the growth attributes were recorded.

2.7. Measurement of Photosynthetic Attributes

Stomatal conductance, transpiration rate, chlorophyll content, chlorophyll fluorescence, and photosynthetic rate were measured. The chlorophyll content was measured using a CCM300 chlorophyll content meter (CCM300, Opti-Sciences, Hudson, NH, USA); chlorophyll fluorescence was measured using an Os5p chlorophyll fluorescence meter (Os5p, Optisciences, Hudson, NH, USA), and photosynthesis by using a portable photosynthesis infrared gas analyzer (LCproT, ADC, Herts, UK).

2.8. Statistical Analysis

All experimental treatments were independently conducted in triplicate, and the mean values were compared using Duncan's multiple range test (DMRT) at $p \leq 0.05$, and a Student's *t*-test. SAS 9.1 (Statistical Analysis System (SAS) software was used for DMRT analysis and GraphPad Prism (San Diego, CA, USA) software version 6.0 was used for the graphical presentation of results.

3. Results

3.1. Isolation and Identification of Plant Growth-Promoting Rhizobacteria

To identify the most reliable phytohormone-producing rhizobacteria showing photosynthetic activity, we collected 14 paddy soil samples and streaked them on LB agar media until pure and clean colonies were obtained. For further investigation, we randomly selected nine bacterial isolates (SIR01, SIR02, SIR03, SIR04, SIR05, SIR06, SIR07, SIR08, and SIR09) to screen for their ability to promote growth in sesame seedlings. As shown in Table 1, SIR01, SIR03, and SIR07 significantly increased the sesame seedling shoot length by 23.3%, 24.3%, and 20.5%, respectively, while enhancing the fresh weight by 18.4%, 20.2%, and 18.8%, respectively, compared to the control plants (non-inoculated). Similarly, SIR08 was not significantly different from the control plants, where SIR02, SIR05, and SIR09 showed moderate differences compared to the control plants (Table 1).

Table 1. Screening bioassay of nine bacterial strains for growth promoting activity in sesame seedlings.

Strains	FW(g)	SL(cm)	Strains	FW(g)	SL(cm)
d.H_2O	11.2 ± 0.08^{b}	1.5 ± 0.54^{c}	SIR05	10.2 ± 0.04^{bc}	1.6 ± 0.39^{b}
SIR01	15.1 ± 0.02^{a}	2.1 ± 0.24^{b}	SIR06	9.8 ± 0.08^{c}	1.3 ± 0.14^{c}
SIR02	10.3 ± 0.05^{c}	1.6 ± 0.30^{c}	SIR07	14.8 ± 0.05^{a}	2.2 ± 0.38^{a}
SIR03	15.3 ± 0.05^{a}	2.4 ± 0.46^{a}	SIR08	11.6 ± 0.06^{b}	1.5 ± 0.10^{b}
SIR04	9.5 ± 0.04^{cd}	1.3 ± 0.37^{d}	SIR09	10.5 ± 0.05^{bc}	1.5 ± 0.32^{b}

The d.H_2O represents seedlings treated with autoclaved distilled water; SIR01–SIR09 represents different bacterial isolates, where the fresh weight (FW) was measured in grams (g) and shoot length (SL) in centimeters. The values represent the mean ± standard deviation of three replicates. Values indexed with the same letter represent non-significant differences between treatments evaluated by Duncan's multiple range test at $p < 0.05$.

3.2. Bacterial Isolation and Initial Screening for Waito-C Rice

Based on their growth-promoting ability on sesame seedlings, we selected three strains, SIR01, SIR03, and SIR07, for further evaluation of their GA production ability. For this evaluation, we used GA-deficient Waito-C rice. The SIR03 isolate significantly enhanced shoot length by 18.1%, root length by 26.4%, and fresh weight by 14.5% compared to control plants (non-inoculated), while SIR01 and SIR07 did not differ significantly compared to control plants (Table 2, Figure 1). This suggests that SIR03 more effectively enhanced the accumulation of GAs than other strains. Based on these observations, the ability of SIR03 to produce GAs was further confirmed by GC/MS. The results indicated that SIR03

synthesized more bioactive GAs. As shown in Figure 2, the SIR03 strain significantly enhanced bioactive GA4, while showing partial accumulation of GA24, followed by GA34 and GA9, compared to the control (only LB broth) (Figure 2).

Table 2. Bioassay for growth promotion activity of three selected strains (SIR01, SIR03, and SIR07) in Waito-C rice.

	Shoot Length	Root Length	Fresh Weight (g)
Control	3.2 ± 0.25^{b}	5.1 ± 0.27^{b}	0.10 ± 0.012^{b}
SIR01	3.3 ± 0.31^{b}	4.8 ± 0.96^{b}	0.10 ± 0.011^{b}
SIR03	4.5 ± 0.31^{a}	8.1 ± 0.56^{a}	0.17 ± 0.012^{a}
SIR07	3.3 ± 0.21^{b}	5.4 ± 0.34^{b}	0.11 ± 0.013^{b}

The control represents non-inoculated seedlings treated with autoclaved distilled water; SIR01, SIR03, and SIR07 represent different bacterial isolates, where the fresh weight was measured in grams (g) and shoot/root length in centimeters. The values represent the mean ± standard deviation of three replicates. Values indexed with the same letter represent non-significant differences between treatments evaluated by Duncan's multiple range test at $p < 0.05$.

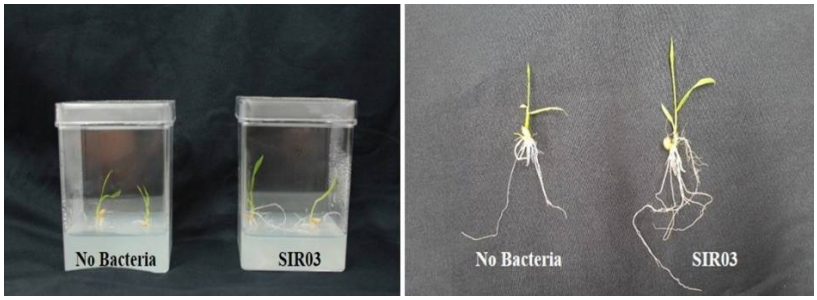

Figure 1. Effects of isolate SIR03 on the growth of Waito-C rice (GA deficient).

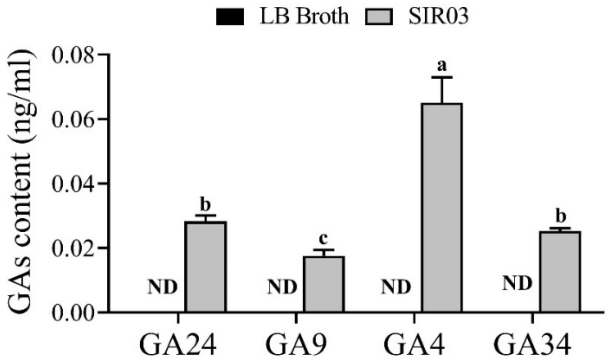

Figure 2. Gibberellin production capacity of isolate SIR03 compared to only LB broth (without any bacteria). The values are means ± standard deviations of three replicates. Results indexed with different letters represent significant differences between different GAs, while ND represents not detected, evaluated by Duncan's multiple range test at $p < 0.05$.

3.3. Identification of SIR03 Strain

Isolate SIR03 led to significant improvement in sesame seedling growth and GAs production until the end of observation; therefore, SIR03 was molecularly identified by amplifying and sequencing 16S rRNA. The identification results showed that the closest identity of 95% was to the *Rhodobacter* sp. FN543495, and with 74% identity to the *Rhodobacter sphaeroides* species, which further shows a close 82% identity of isolate SIR03 to *Rhodobacter sphaeroides* AB196354. This suggests the isolate SIR03 has the closest similarity to the *Rhodobacter sphaeroides/Rhodobacter* species (Figure 3). The obtained nucleotide sequences were submitted to the NCBI GenBank database with accession no. MW345829.

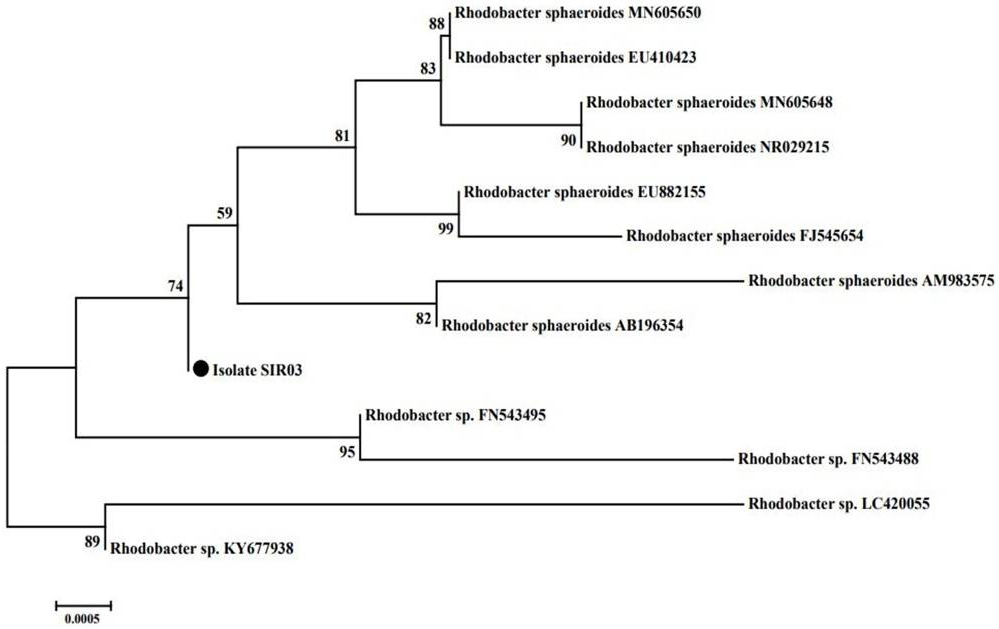

Figure 3. Phylogenetic tree of ALT1, which was constructed using 16S rRNA sequences by neighbor-joining and maximum likelihood methods.

3.4. Isolate SIR03 and Biochar Promote Sesame Growth

After the above screening and bioassay, we evaluated the effects of the SIR03 strain along with biochar on sesame seedling growth and development. The results showed that solely biochar-treated plants had non-significant differences in the root, shoot, and biomass of sesame seedlings compared to control plants, whereas solely inoculation with SIR03 strain improved the sesame seedling shoot length and fresh weight by 13% and 28%, respectively, and root length and fresh weight by 20.7% and 19.8%, respectively, compared to control plants (non-inoculated). However, the combined application of biochar and SIR03 strain showed more dominant increases in shoot length and fresh weight, by 15.8% and 30.7%, respectively, and root length and fresh weight, by 28.8% and 26.2%, respectively, compared to control plants (non-inoculated). These results suggest a positive relationship between the SIR03 strain and biochar, and that their combined application significantly improved plant growth and development (Table 3).

Table 3. Effects of isolate SIR03 on growth and biomass of sesame seedling.

	Shoot Length	Root Length	Shoot F.W (g)	Root F.W (g)
Control	24.6 ± 1.25^{b}	11.1 ± 0.94^{c}	6.05 ± 0.654^{b}	1.56 ± 0.065^{c}
Biochar	25.5 ± 1.95^{b}	13.5 ± 1.04^{b}	6.55 ± 0.423^{b}	1.88 ± 0.042^{b}
R. Sphaeroides SIR03	27.8 ± 1.03^{ab}	13.4 ± 0.93^{b}	7.75 ± 0.214^{a}	1.87 ± 0.038^{b}
Biochar + *R. Sphaeroides* SIR03	28.5 ± 0.52^{a}	14.3 ± 0.51^{a}	7.91 ± 0.131^{a}	1.97 ± 0.070^{a}

Control represents the non-inoculated seedlings treated with autoclaved distilled water; the shoot length is in centimeters, root length in centimeters, F.W is the fresh weight (g). The values represent the mean ± standard deviation of three replicates. Values indexed with the same letter represent non-significant differences between treatments, evaluated by Duncan's multiple range test at $p < 0.05$.

3.5. Isolate SIR03 and Biochar Improved Transpiration Rate and Stomatal Conductance of Sesame Seedlings

Transpiration is an important factor in enabling plants to maintain water absorption for a healthy life cycle. As the transportation rate increases, water absorption increases, and the stomata opening and closing in response also have effects. The current results

show that the sole biochar significantly improved the transpiration rate by 30.7% and stomatal conductance by 31.8% compared to control plants (non-inoculated); conversely, the combined application of the SIR03 strain and biochar caused further increases in the transpiration rate and stomatal conductance, by 48.1% and 54.5%, respectively, compared to control plants, while showing non-significant differences compared to sole SIR03 treated plants (Figure 4A,B).

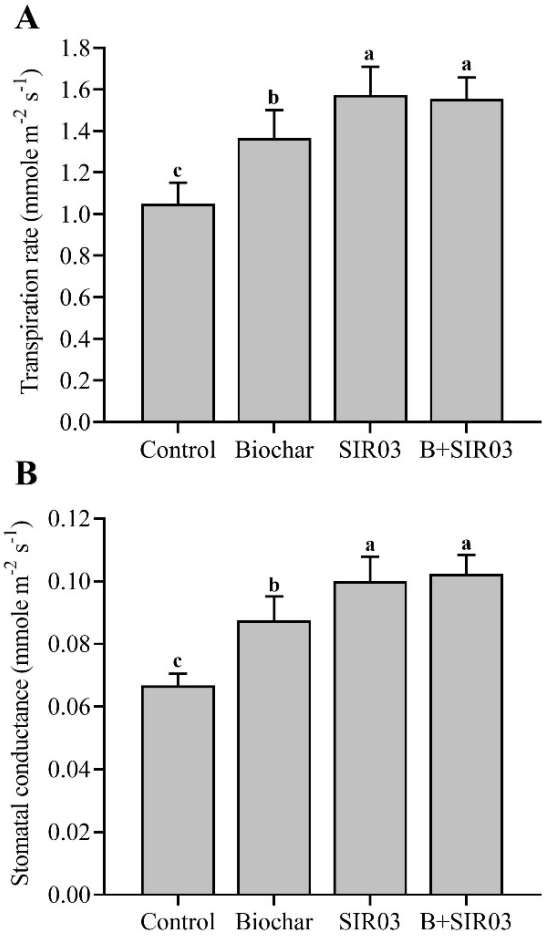

Figure 4. Effects of isolate SIR03 on (**A**) transpiration rate and (**B**) stomatal conductance in sesame seedlings. Control represents (non-inoculated); Biochar represents biochar treated; SIR03 is the bacterial isolate; B + SIR03 represents the combined application of biochar and SIR03 strain. The values are means ± standard deviations of three replicates. Values indexed with different letters represent significant differences between treatments, evaluated by Duncan's multiple range test at $p < 0.05$.

3.6. Isolate SIR03 and Biochar Improved Photosynthetic Rate and Chlorophyll Fluorescence of Sesame Seedlings

The results showed that the photosynthetic rate under the sole biochar treatment did not differ significantly from control plants (non-inoculated), whereas the treatment with the sole SIR03 strain caused a significant increase in photosynthetic rate, by 11.6% compared to control plants (non-inoculated). However, the combined application of SIR03 and biochar caused a significant increase in the photosynthetic rate by 14.4% compared to control plants (non-inoculated), but showed a non-significant difference compared to sole SIR03 inoculated plants (Figure 5A). In the case of chlorophyll fluorescence (Fv/Fm), no significant differences were found between the sole biochar, sole SIR03, and the combined application of SIR03 and biochar, compared to the control plants (non-inoculated) (Figure 5B).

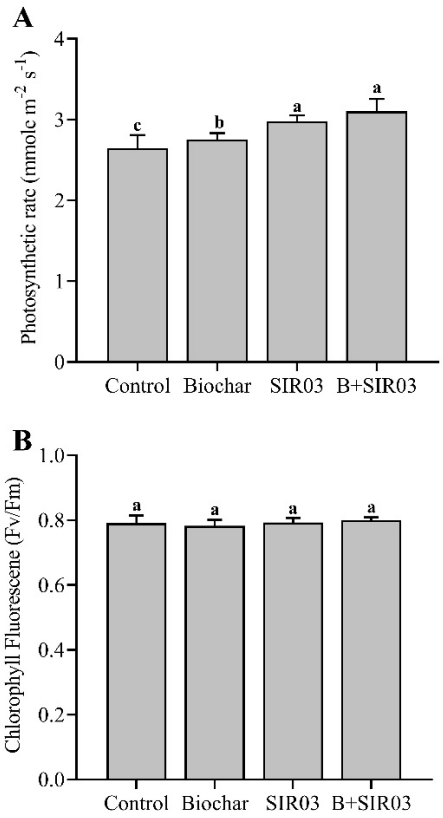

Figure 5. Effects of isolate SIR03 on (**A**) photosynthetic rate and (**B**) chlorophyll fluorescence in sesame seedlings. Control represents the (non-inoculated); Biochar represents biochar treated; SIR03 is the bacterial isolate; B+SIR03 represents the combined application of biochar and SIR03 strain. The values are means ± standard deviations of three replicates. Values indexed with different letters represent significant differences between treatments, evaluated by Duncan's multiple range test at $p < 0.05$.

4. Discussion

Biofertilizers are emerging biological tools used to promote sustainable agriculture and regulate plant growth and development to combat environmental threats. In the present study, we tried to elevate the vital role of rhizobacterium SIR03 along with the application of biochar in sesame seedlings and to investigate the ability of SIR03 to regulate various metabolic aspects, such as the functioning of endogenous phytohormones and improvements in plant growth and photosynthetic ability. It is well known that due to increased environmental pollutants and contamination, the soil loses its fertility, and plants cope with abnormal growth, which affects yield and productivity. Many studies have reported different phytohormone-producing and growth-stimulating rhizobacterial strains that respond to different environmental conditions [48,49]. The current study investigated the GA-producing SIR03 strain, which improved the growth of Waito-C rice (a GA-deficient), to demonstrate its GA-producing ability.

Similarly, plant growth-promoting rhizobacteria and biochar are vital tools for improving plant growth and development. Biochar provides a favorable environment in soil for sustaining microbiomes and facilitates the symbiotic association of PGPR in the plant rhizosphere [50]. Many researchers have reported the production of a wide range of secondary metabolites and the maintenance of nutrient recycling in the presence of these amendments [33,51,52]. Similarly, the current results show that inoculation of SIR03 in combination with biochar significantly enhanced seedling characteristics by improving plant growth and biomass, including fresh and dry weight and facilitated transpiration and stomatal conductance. A report from Chen et al. (2018) [53] showed that biochar

significantly improved plant growth and the microbial community, and increased moisture, temperature, and nutrients in green roof substrates. Similarly, the combined use of biochar and PGPR enhanced soil physicochemical properties and moisture content, which further facilitated the improvement of plant photosynthesis, stomatal conductance, and relative water content, and enhanced the uptake of K+ and K+/Na+ [51]. This may be correlated to the ability of PGPR to promote plants' ability to produce phytohormones such as GAs, which improve plant–microbial interactions [52]. Plant–microbial interactions are very important in mitigating stress and enhancing growth-promoting characteristics, modulating intermediate metabolites that enhance plant growth, and regulating various developmental processes from germination to harvesting. Various recent studies have shown that microbes can mediate the production of phytohormones, such as gibberellic acid, which enhances the significant growth of plants even under abiotic stress [54,55], while biochar is a carbon-rich thermal organic compound that acts as a natural carbon supplier with no side effects unlike those of agrochemicals [56,57].

Furthermore, the addition of biochar along with PGPR resulted in longer periods of moisture content, which may be due to a reduction in soil bulk density and holding water capacity, which in turn increases the availability of mineral nutrients [58,59]. The synergistic effect of PGPR and biochar further increased total bacterial abundance owing to a biostimulation impact [60], and augmented the microbial activities in the rhizosphere, ultimately resulting in better plant growth. In the past, many agrochemicals have been developed to enhance seedling characteristics, but their application has huge side effects, so it is urgent to introduce a new pathway with minimal side effects [61]. The present study combined the application of biochar, a natural compound that provides carbon and improves nutrient supply, and microbial inoculation, which promotes the production of gibberellic acid which acts as a signaling molecule. There are increased photosynthetic, stomatal characteristics, and the improved quality of seedlings were observed, suggesting that the combined application of biochar and SIR03 can enhance growth and photosynthetic characteristics, which can improve the plant development under field capacity.

5. Conclusions

The synergistic use of biochar and PGPR led to improved physiochemical properties and physiological characteristics such as plant growth and biomass. Biochar and PGPR can be a potential tool to improve plant photosynthesis, transpiration, and stomatal conductance. The synergistic use of PGPR (SIR03) and biochar, therefore, can enhance the growth and productivity of sesame plants under field capacity in different environmental conditions. Therefore, the application of biochar and SIR03 can be considered eco-friendly and environmentally safe bio-fertilizers. This study could provide a potential strategy for increasing soil fertility and plant productivity and achieving sustainable agriculture.

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