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ARTICLE

Pseudomonas veronii KJ mitigates flood stress-associated damage in *Sesamum indicum* L.

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Abstract Physiological characteristics of terrestrial plants are severely affected by waterlogging stress, leading to low photochemical efficiency of leaves and retarded growth and development. Plant growth-promoting rhizobacteria contain the *acdS* gene, which encodes for the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. ACC deaminase cleaves the substrate ACC to produce α-ketobutyrate and ammonia and mitigates the adverse effect of prolonged water stress. The aim of this study was to characterize ACC deaminase-producing rhizobacteria and evaluate their effects on sesame (*Sesamum indicum* L.) under waterlogging stress condition. The rhizobacterium *Pseudomonas* KJ was characterized on the basis of sequencing of the partial 1501 bp fragment of 16S rDNA amplicon and confirmed as *Pseudomonas veronii* KJ. ACC-supplemented minimal medium revealed the phenotypic identification of *acdS* gene. The nucleotide sequence (1001 bp) of ACC deaminase gene of *P. veronii* KJ was also confirmed. We used *P. veronii* KJ as a bioinoculant in waterlogging stress and monitored the growth and developmental characteristics of sesame plants, including leaf chlorophyll fluorescence signals, concentration of chlorophyll, root and shoot length, and fresh and dry biomass in stressed versus unstressed plants. Plants treated with *P. veronii* KJ significantly ($P \leq 0.05$) mitigated the waterlogging stress-related damage. Thus, the rhizobacterium *Pseudomonas veronii* KJ may be considered as a commendable addition to the consortium of beneficial microbes for its ability to reduce waterlogging stress-related damage in sesame plants.

Keywords ACC deaminase-producing and plant growth-promoting rhizobacteria · *acdS* gene · *Pseudomonas veronii* KJ · *Sesamum indicum* L. · Waterlogging stress

Introduction

Sesame (*Sesamum indicum* L.) is one of the most ancient cultivated oil crops in Asia and increasingly grown worldwide [1]. China, India, Nigeria, Tanzania, Myanmar, and Sudan are the top producers of black and white sesame and contribute to more than 60% of annual global supply of sesame seeds [2]. Sesame seeds may be directly used in different food items, as these contain 33–58% oil and 15–30% proteins. In addition, sesame seeds are known to exhibit various therapeutic compounds, including antioxidants (sesamin, sesamolin, sesaminol, and sesamolinol) and dietary fibers [2, 3]. Optimization of cultivation factors such as water, fertilizers, and all biotic and abiotic stress conditions is essential to achieve high yields of sesame [4]. In particular, waterlogged condition during the early stage of cultivation is shown to hamper the growth and productivity of crops by up to 30% [1, 4]. Land plants become hypoxic or are oxygen deprived in waterlogged conditions, owing to the decrease in the oxygen diffusion into roots [5, 6]. As healthy plants need soil oxygen concentrations above 0.1 L/L, waterlogged conditions may decrease the amount of oxygen, leading to the production of ethylene and accumulation of toxic compounds [5–7].

The gaseous hormone ethylene is found in all higher plants and greatly contributes to normal growth and

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development. In addition, ethylene is a key compound that plays an important role in the response of plants to a variety of stress conditions [8]. Under hypoxic condition, plants produce increased level of the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) synthase that converts the substrate *S*-adenosyl-L-methionine (SAM) to ACC, whereas the study of Lee et al. [9] demonstrated that recombinant ACC synthase can also convert SAM into ACC for the production of ethylene. Upon oxidation, the substrate ACC is converted to ethylene by ACC oxidase [8, 10]. Higher concentrations of ethylene (stress ethylene) in plant tissues may adversely affect the physiological response of plants and initiate senescence, chlorosis, leaf abscission, growth inhibition, or even death [11].

Plant growth-promoting bacteria (PGPR) use different mechanisms to mitigate the damage induced by waterlogged stress and promote plant growth under stress conditions [12]. The bacteria found in near vicinity of plant roots and producing the enzyme ACC deaminase are of great importance. ACC deaminase cleaves ACC into ammonia and α-ketobutyrate and reduces the level of stress ethylene [13]. Thus, bacterial ACC deaminase may play an important role by reducing the extremely high level of ethylene and alleviating damages caused by waterlogged stress in plants [8, 10].

Environmental stress conditions directly affect the photosynthetic machinery of plants; hence, fluorescence signals are widely used to estimate the activity of photosystem II under different environmental stress conditions [14]. The available literature strongly focuses on the use of chlorophyll fluorescence as a suitable tool for the assessment of environmental fitness and monitoring of photosynthetic events at different levels under abiotic stress conditions [14, 15]. Plants respond to oxygen deficiency at different levels from the activation of signal transduction pathways to metabolic adaptation, leading to morphological changes and activation of other related strategies [16, 17]. Several studies have demonstrated the adverse effect of waterlogging stress on major physiological functions of plants, and reduction in the concentration of chlorophyll, stomatal conductance, and biomass decline are the primary effects of waterlogging stress. These are often accompanied with the reduced CO_2 assimilation, which confers an adverse effect on the growth and development of plants [15, 18].

To our knowledge, no study has attempted to study the effect of PGPR in sesame. However, the use of ACC deaminase-producing bacteria in waterlogged condition may significantly improve the concentration of chlorophyll and photochemical efficiency of leaves and increase the biomass of sesame plants.

Materials and methods

Bacterial isolation and identification

The rhizospheric bacterial strain (KJ) was isolated from melon fields in Seongju, Republic of Korea. Morphological observations were reported based on the colony characteristics, and presumptive identification was performed on the basis of Bergey's Manual of Determinative Bacteriology [19]. For more efficient screening, the rhizobacterial isolate was purified by streaking several times onto nutrient agar plates and different tests such as oxidase, catalase, and Gram staining were performed. The selected rhizobacterial isolate was stored at 4 °C for further use.

Screening of the bacterial isolate for the utilization of ACC as a nitrogen source

The bacterial isolate was screened for its ability to utilize ACC as a sole nitrogen source in Dworkin and Foster (DF) minimal medium [20] according to the methods of Penrose and Glick [21] with some modifications. Two sets of DF minimal medium plates were prepared for the phenotypic identification of *acdS* gene. One set of plates was supplemented with ammonium sulfate ($(NH_4)_2SO_4$, 2 g/L), while the other set comprised a lawn of the substrate ACC (3 mM). The selected bacterial culture was inoculated on to the medium plates and incubated at 28 °C for 4–5 days.

Direct amplification of 16S rDNA, sequencing, and homology analysis

For the direct amplification of 16S rDNA, the pure rhizobacterial strain was cultured on Luria–Bertani (LB) agar medium plate for 20–24 h at 28 °C. Amplification of 16S rDNA was carried out according to the method of Giovannoni et al. [22] with some modifications using a thermal cycler (Bio-Rad T100^{TM}). The sequences of the oligonucleotide primers used for the amplification of 16S rDNA were as follows:

Forward primer 27F (5′AGAGTTTGATCMTGGCTC AG3′)

Reverse primer 1492R (5′TACGGYTACCTTGTTACG ACTT3′)

Polymerase chain reaction (PCR) of 16S rDNA from the isolate (positive for ACC deaminase activity) was performed in a final volume of 50 µL. The reaction mixture comprised 43 µL of ultrapure water, 5 µL of 10× PCR buffer, 1.0 µL (10 mM) of each dNTP (dATP, dCTP, dGTP, and dTTP), 0.3 µL (10 µM) of forward primer (27F), 0.3 µL (10 µM) of reverse primer (1492R), and 0.75 µL (2.5 U/µL) of *Taq* DNA polymerase. A single

colony of the isolate (KJ) was picked up with a sterilized toothpick into a PCR reaction tube containing PCR reagents for the amplification of 16S rDNA. Amplification of 16S rDNA was performed in a thermal cycler (Bio-Rad T100^{TM}) using an initial denaturation at 94 °C for 5 min (one cycle), followed by 35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min and a final extension at 72 °C for 5 min (one cycle). In addition, no template control (NTC) reaction without any bacterial colony was used as a negative control. The amplified product was run on a 1.2% agarose gel along with the marker at a constant voltage and visualized under UV light. The product was stored at 4 °C.

The amplified and purified PCR product was sequenced by SOLGENT, Korea. Partial sequence of 16S rDNA of the rhizobacterial isolate was obtained after sequencing. The sequences were compared using BLAST search program for sequence homology of the nucleotides. Closely related sequences were aligned through CLUSTALW using MEGA 6.0 software [23]. The same software was used for the construction of maximum parsimony (MP) tree. Bootstrap replications (1000) were used for the nodes in the phylogenetic tree.

Quantification of ACC deaminase activity

The bacterial isolate was cultured in DF medium for 20 h at 28 °C in a shaker incubator at 200 rpm, and ACC deaminase activity was assayed according to the method of Penrose and Glick [21] with slight modifications. The enzyme ACC deaminase cleaves the substrate ACC to produce α-ketobutyrate, which is determined spectrophotometrically at 540 nm wavelength. The rhizobacterium was inoculated into DF media containing different concentrations (0, 1, 2, and 3 mM) of ACC. The amount of α-ketobutyrate (nmol) produced was measured by comparing the absorbance of the sample with the values from the standard curve of α-ketobutyrate. A stock solution of α-ketobutyrate (Sigma Aldrich Co.) was already prepared in Tris–HCl (0.1 M, pH 8.5) and stored at 4 °C.

Plant growth conditions

White sesame seeds were surface sterilized with 75% ethanol for 2 min and thoroughly washed with deionized distilled water to remove any attached debris. The sterilized seeds were sown in pots (10.0 × 10.0 × 8.5 cm) packed with commercially available horticultural soil (Biosento-1, Hungnong-jongmyosa Company, Republic of Korea). These pots were placed in a growth chamber at the laboratory of Biomass and Life Chemistry, Kyungpook National University (KNU), Republic of Korea. Temperature and relative humidity were maintained at 25 ± 5 °C and 60%, respectively. Light intensity was maintained at 650 μmol m^{-2} s^{-1} and 16/8 h light/dark cycle for 4 weeks without bacterial inoculation and waterlogging stress.

Bacterial inoculation and waterlogging treatment

Bacterial inoculum was prepared by growing ACC deaminase-producing bacteria (KJ) in LB nutrient broth at 28 °C for 24 h in a shaker incubator at 200 rpm until the cells reached their late log (exponential) phase. To obtain a uniform population of bacterial inoculum (10^8 colony-forming units [CFU]/ml), the optical density of the culture was measured before inoculation. Bacterial culture was centrifuged at 8000×g for 10 min at 4 °C. The pellet was washed thrice with distilled water and resuspended in sterile distilled water. The absorbance of the bacterial suspension was adjusted (A_{600nm} = 1.0) before its inoculation in 50 mL of suspension (just once during the experiment) of sesame plants grown under growth chamber. The inoculation process was carried out 1 week prior to the application of waterlogging stress. For control, only distilled water (50 mL) was applied to the seedlings. The seedlings were subsequently subjected to waterlogging stress by placing the pots under submerged conditions in a water tank. Water level was maintained up to the surface of the soil. A total of 10 days of waterlogging was applied to the seedlings. On the other hand, a set of seedlings was grown in the original growth condition without water stress (control).

Measurement of growth parameters

After 10 days of waterlogging stress, the different growth variables, including, height (cm), root length (cm), and fresh and dry biomass (g), were measured. The seedlings were weighed immediately after harvesting to obtain fresh biomass, while dry biomass was measured after oven drying at 70 °C overnight.

Chlorophyll content

The concentration of chlorophyll was measured at different time points (0-day post-flooding [0-DPF], 2-DPF, 4-DPF, and 8-DPF) from the commencement of waterlogging treatment to just before the end of the experiment. For accurate and easy determination of chlorophyll content, CCM-300 (Opti-Sciences) chlorophyll content meter was used. The working principal and design of CCM-300 is based on the work of Gitelson [24] using the ratio of fluorescence at 735/700 nm to determine chlorophyll content. The results were deciphered as relative chlorophyll content in mg/m^2. The chlorophyll content of the same seedling in each treatment was measured in triplicate using the third and fourth most recent and fully expanded leaf.

Photosynthesis analysis

Photosynthesis yield analyzer (Mini-PAM, Effeltrich, Germany) was used to calculate the electron transport rate (ETR) resulting from the rapid light curve (RLC) measurements. Photosynthesis analysis of leaves from each treatment was recorded at room temperature. The leaf clip holder (Walz, Effeltrich, Germany, 2030-B) was connected to the photosynthesis yield analyzer, which was also connected to a computer with data acquisition software (Walz, DA-2000). All required measurements were taken with the saturation pulse method according to the manual of Mini-PAM. Mini-PAM accounts for all applicable fluorescence parameters as well as actinic irradiance and leaf temperature and estimates ETR. Parameters such as ETR and photosynthetically active radiation (PAR) were calculated for the third and fourth leaf (lamina region) of the plant. ETR was calculated as follows:

$$\mathrm{ETR} = \mathrm{Yield} \times \mathrm{PAR} \times 0.5 \times \mathrm{AF}$$

where yield shows the whole amount of photochemical quantum ($Y = [F'm - F']/F'm$), 0.5 is the constant of photon energy absorbed by photosystems (I and II) during sampling under in situ condition, and AF is the constant (estimated as 0.84) that represents the absorption factor of thallus. Three measurements were taken per pot and two pots per treatment, while the total number of treatments was four.

Data analysis

The study included four treatments, and each treatment had three replicates in a completely randomized design. Treatment effects were determined with the analysis of variance followed by *t* test at a probability level of $P = 0.05$.

Results

Bacterial identification and screening for the utilization of the substrate ACC

The rhizobacterial isolate obtained from the melon field in Seongju, Republic of Korea, was subjected to possible identification based on the Bergey's Manual of Determinative Bacteriology [19]. The strain was identified as a gram-negative, short rod-shaped bacterium with circular colonies. The isolate also presented positive results for both oxidase and catalase tests. In addition, the isolate was tested for substrate ACC utilization on DF minimal medium supplemented with the substrate ACC or $(NH_4)_2SO_4$, and appropriate bacterial growth was recorded on $(NH_4)_2SO_4$-containing plates and ACC (3 mM)-supplemented plates after 5 days of incubation at 28 °C (Fig. 1). The bacterial growth on ACC-supplemented plates revealed the phenotypic identification of *acdS* gene that encodes for ACC deaminase, which utilizes the substrate ACC as a sole source of nitrogen.

The rhizobacterial isolate *Pseudomonas* KJ was evaluated with molecular methods for its precise identification. Molecular characterization based on 16S rDNA sequencing of the partial sequence (1501 bp) of the isolate *Pseudomonas* KJ was compared with that from the database by BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences from direct amplification of 16S rDNA and MP analysis revealed that the strain was closely related to *Pseudomonas veronii*, while BLASTn search showed 99% homology (Fig. 2). Thus, the rhizobacterium *Pseudomonas* KJ was characterized on the basis of sequencing (sequences not shown here) of 16S rDNA amplicon and confirmed as a strain of *P. veronii* KJ represented in the phylogenetic tree (Fig. 2).

Amplification and sequencing of acdS gene and ACC deaminase activity determination

After confirming the phenotypic identification of *acdS* gene on minimal medium, specific primers were designed for the amplification of *acdS* gene of the rhizobacterium *P. veronii* KJ. The identified strain yielded a 1001 bp DNA fragment (sequence not shown) and gene sequencing and BLASTn search analysis confirmed the presence of *acdS* gene in *P. veronii* KJ. Different concentrations of ACC were used for the quantification of ACC deaminase activity of *P. veronii* KJ. Higher level of (979 μM h^{-1} mg^{-1}) of α-ketobutyrate was measured at higher concentration (3 mM) of ACC in the growth media, while the level of α-ketobutyrate produced gradually decreased with a decrease in the concentration of ACC (Fig. 3). Together these data indicate the presence and expression of ACC deaminase in *P. veronii* KJ.

Effect on sesame growth and biomass

The screening results presented in Fig. 4 and Table 1 show that the application of ACC deaminase-producing *P. veronii* KJ resulted in a significant decrease ($P \leq 0.05$) in waterlogging stress-associated damage in sesame seedlings. The root length, shoot length, fresh biomass, and dry biomass significantly increased following treatment with *P. veronii* KJ under waterlogging stress condition (Table 1). The advantages of bioinoculation on the growth of sesame seedlings were observed in the pot experiment, wherein the seedlings demonstrated the potential role of ACC deaminase-producing *P. veronii* KJ under waterlogged condition (Fig. 4).

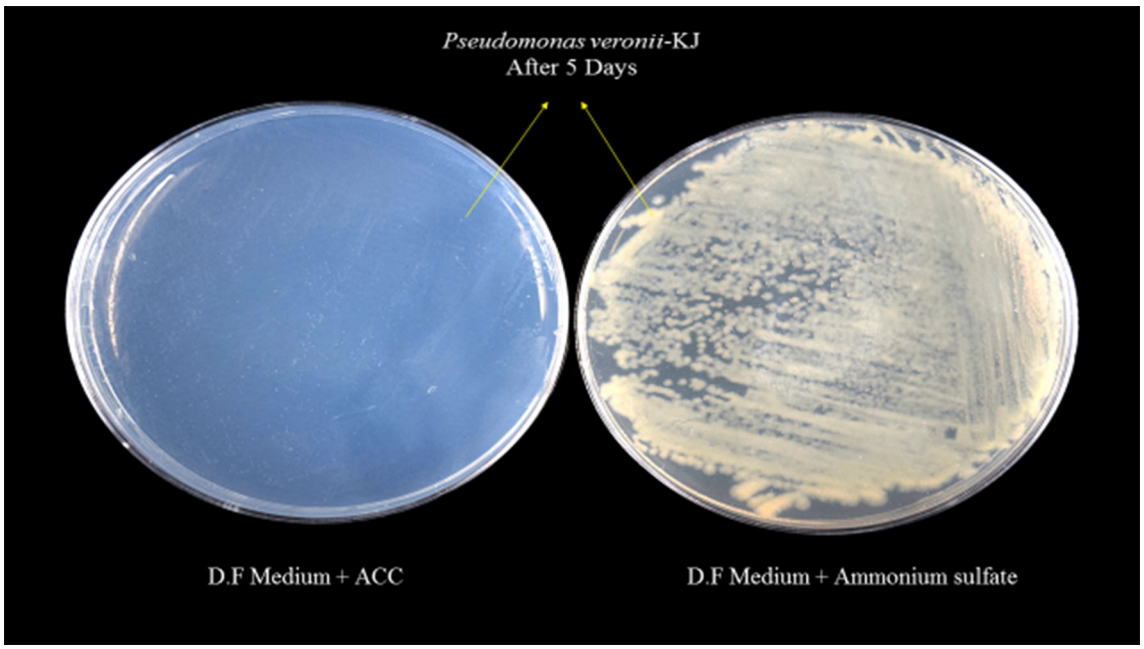

Fig. 1 Screening of *Pseudomonas* KJ for the utilization of ACC on DF medium. The minimal medium plates were supplemented with ACC (3 mM) or ammonium sulfate (2 g/L). Bacterial growth was recorded on both ammonium sulfate-containing and ACC-supplemented plates after 5 days of incubation at 28 °C

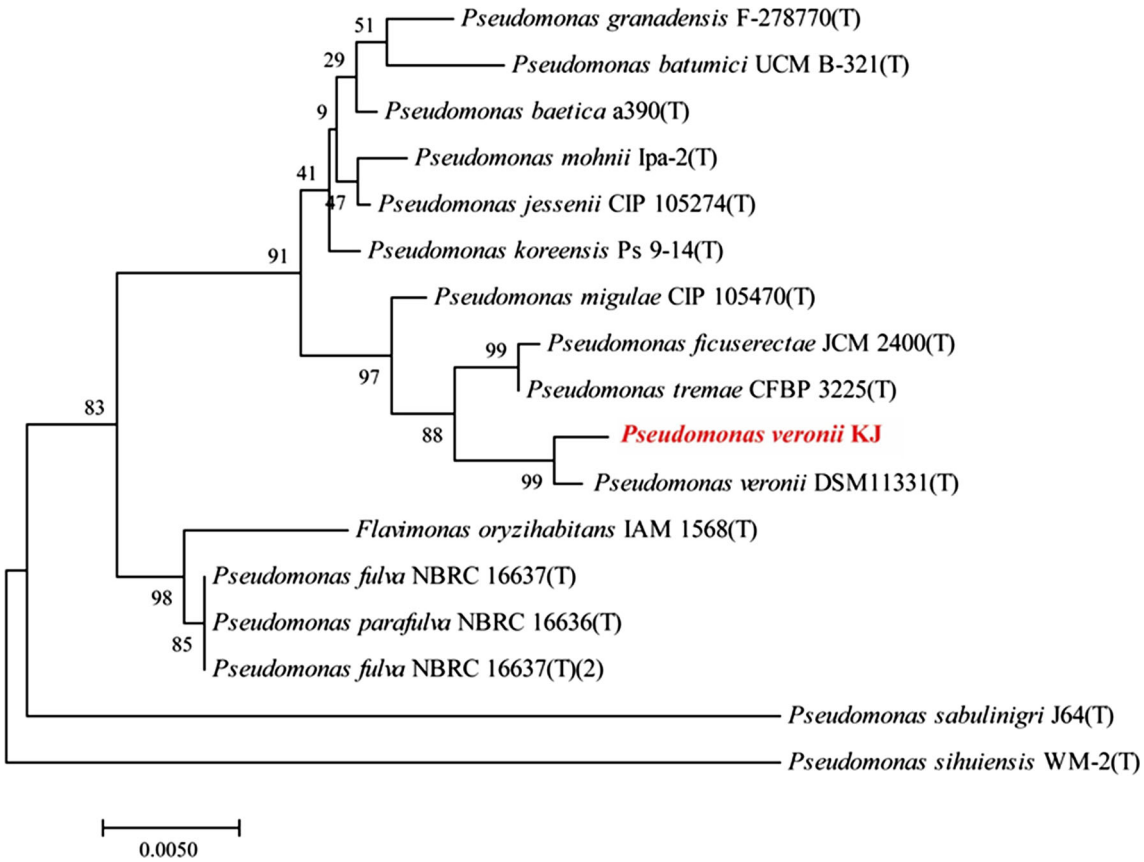

Fig. 2 Phylogenetic analysis of *P. veronii* KJ using 16S rDNA partial sequences. The genetic relatedness between *P. veronii* KJ and other members of *Pseudomonas* species was analyzed using MEGA 6.0 software, and the same software was used for the construction of maximum parsimony tree. The nodal robustness of the tree was assessed using 1000 bootstrap replicates

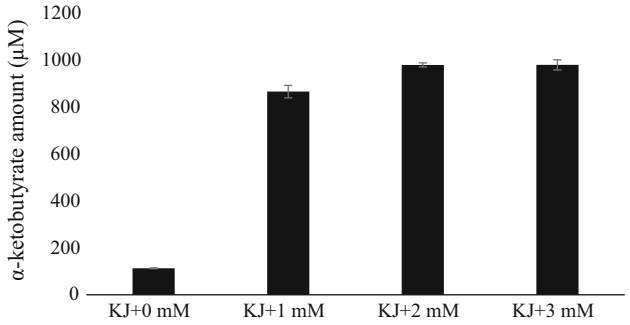

Fig. 3 ACC deaminase activity of *P. veronii* KJ in DF minimal medium supplemented with different concentrations (0, 1, 2, and 3 mM) of the substrate ACC. The amount of α-ketobutyrate (μM) produced was directly proportional to the concentration of ACC in the media

The prominent adverse effect of waterlogging stress was observed on the shoot length of sesame plant that decreased by about 41% as compared to unstressed control plants. However, ACC deaminase-producing bacteria significantly ($P \leq 0.05$) improved the shoot length of plants under waterlogged condition, as observed from up to 21% increase in the length of treated plants as compared with that of untreated plants in waterlogged condition. Although bacteria-treated plants in the absence of waterlogging stress showed improved shoot length as compared with unstressed control plants, the difference was insignificant (Table 1). Vigorous growth of adventitious roots was observed in bacteria-treated plants, highlighting the capability of sesame plants to survive under waterlogging condition. Furthermore, substantial difference was recorded between the root length of control and stressed plants; plants treated with bacteria showed robust growth and noticeable increase in root length as compared with stressed plants. The root length of plants in waterlogged condition was significantly reduced ($P \leq 0.05$) by 51.6% as compared with the root length of control plants. On the contrary, plants treated with *P. veronii* KJ under waterlogging condition showed 28.93% increase in the root length as compared with untreated flooded plants. *P. veronii* KJ significantly increased the fresh and dry biomass of plants under flooding conditions. Hence, ACC deaminase-producing *P. veronii* KJ showed prominent results by mitigating the flooding stress-associated damages in sesame plants (Table 1).

Chlorophyll content

Waterlogging condition greatly affects the chlorophyll content of leaves, while the bacteria (producing ACC deaminase) residing in the near vicinity of plant roots may alleviate the damage caused by flooding stress [18]. In our results, the chlorophyll content of the leaves was significantly ($P \leq 0.05$) affected by flooding stress. In comparison with control plants, waterlogged plants showed a prominent reduction in their chlorophyll content. On the other hand, plants treated with *P. veronii* KJ and subjected to waterlogged condition showed a significant ($P \leq 0.05$)

Fig. 4 Morphological comparison of *S. indicum* plants

Table 1 Effect of waterlogging stress on *S. indicum* treated with *P. veronii* KJ

Treatment	S.L. (cm)	R.L. (cm)	F.B. (g)	D.B. (g)
Control	24.5 ± 0.60^{a}	9.1 ± 1.04^{a}	9.22 ± 2.24^{a}	3.15 ± 0.82^{a}
Bacterized	25.5 ± 0.28^{a}	15.6 ± 4.4^{b}	14.18 ± 1.6^{b}	5.31 ± 0.87^{b}
Flooded	14.6 ± 2.32^{b}	4.4 ± 0.51^{c}	3.54 ± 0.23^{c}	0.55 ± 0.15^{c}
Flooded + bacterized	17.8 ± 1.75^{c}	7.03 ± 0.85^{d}	4.00 ± 0.47^{d}	0.82 ± 0.08^{d}

The values in each column represent the mean ± SD

S.L., shoot length; *R.L.*, root length; *F.B.*, fresh biomass; *D.B.*, dry biomass

Those marked with different letters in each column are significantly different at $P \leq 0.05$, as analyzed by *t* test

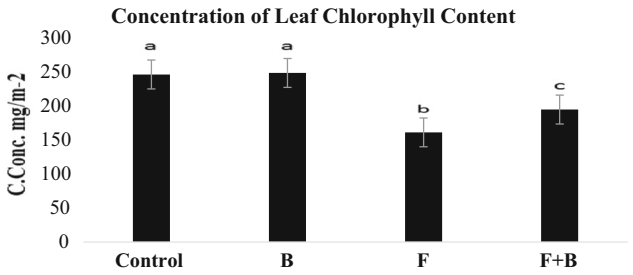

Fig. 5 Effect of flooding stress on the chlorophyll content of *S. indicum* L. leaves upon treatment with ACC deaminase-producing *P. veronii* KJ. The chlorophyll content significantly reduced in flooded plants (*F*) as compared with control. However, plants treated with bacteria showed a significant increase in their chlorophyll content after flooding ($F + B$) stress as compared with stressed plants without bacterial treatment. The unstressed bacteria-treated plants (*B*) and control plants showed no significant difference in their chlorophyll content. Values are presented as mean ± SD (error bars). Different letters over error bars indicate significant differences ($P \leq 0.05$) using *t* test

increase in their chlorophyll content as compared with nontreated plants. Upon induction of flooding stress, a minor increase was observed in the chlorophyll content of flooded plants that dramatically declined thereafter, with more than 30% decrease in the chlorophyll content reported (Fig. 5).

The chlorophyll fluorescence ratio (CFR) was initially more than 0.94 and gradually reduced (0.78–0.94) under flooding condition. CFR of plants from the control group was more than 0.94 until the end of the experiment. Bacteria-treated plants showed higher chlorophyll content and CFR value as compared with the control plants, but no significant ($P \leq 0.05$) difference was noted. On the contrary, plants subjected to flooding stress presented a very low CFR (0.78) value at the end of the experiment as compared to those treated with *P. veronii* KJ in waterlogged condition (Fig. 6).

The content of chlorophyll decreased in waterlogged plants as compared with unstressed plants. On the other hand, waterlogged plants treated with *P. veronii* KJ showed significantly higher chlorophyll level and leaf CFR value at 735/700 nm. Hence, ACC deaminase-producing *P. veronii* KJ mitigates the damage induced by flooding stress on the chlorophyll content and contributes to the growth and development of plants.

Fluorescence yield and RLC

The photosynthetic characteristics of plant leaves were associated with the decrease in the chlorophyll content under waterlogged condition. The ETR value of PS-II presented a negative deviation at the end of experiment (Table 2). Figure 7 and Table 2 show the aptitude of RLC and fluorescence yield, respectively, for sesame leaves under waterlogged condition.

The reducing performance of ETR was more obvious for flooded plants than the control, while plants treated with bacteria under flooded condition showed a minimal decrease in ETR, thereby expressing a reasonable tolerance for waterlogging stress. The decrease in ETR may serve as an indicator of electron transport chain deterioration in PS-II. The photochemical process is more sensitive in sesame plant subjected to flooding stress than that exposed to bacterial treatment, which enables the plant to maintain relatively normal values of ETR under waterlogged condition. All other photosynthetic characteristics were greatly affected in response to flooding stress. Flooded plants showed a very low *Fm* value as compared with unstressed plants, which showed reduced efficiency of heat dissipation

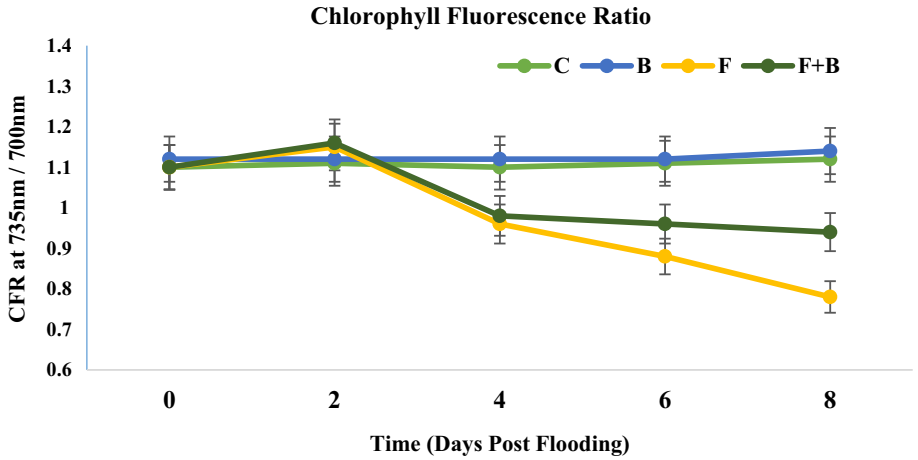

Fig. 6 Effect of flooding stress on the leaf chlorophyll fluorescence ratio at 735/700 nm. *S. indicum* in flooding (*F*) stress condition showed significantly lower CFR value than in control conditions (*C*). However, the non-stressed bacteria-treated plants (*B*) and control plants (*C*) showed no significant difference in CFR value. ACC deaminase-producing bacteria significantly increase the CFR value under flooded condition ($F + B$) at different time points. Values are presented as mean ± SD (error bars)

Table 2 Different parameters of chlorophyll fluorescence after 8 days of flooding stress

Treatment	Y(II)	ETR	ETRm	*Fo*	*Fm′*	*Fm*
Control	0.804 ± 0.005[a]	68.7 ± 10[a]	86.12 ± 3[a]	616.3 ± 39[a]	1990 ± 86[a]	3684 ± 258[a]
Bacterized	0.806 ± 0.002[a]	69.7 ± 7[a]	84.3 ± 2[a]	661.3 ± 7[a]	1858 ± 76[a]	3674 ± 42[a]
Flooded	0.735 ± 0.009[b]	23.9 ± 1[b]	52.8 ± 4[b]	752.3 ± 18[b]	1468 ± 82[b]	2318 ± 96[b]
Flooded + bacterized	0.770 ± 0.002[c]	38.1 ± 4[c]	57.5 ± 3[c]	711.6 ± 3[c]	1704 ± 26[c]	3264 ± 56[a]

Values in each column represent the mean ± SEM and those marked with different letters in each column are significantly different at $P \leq 0.05$, as analyzed by *t* test

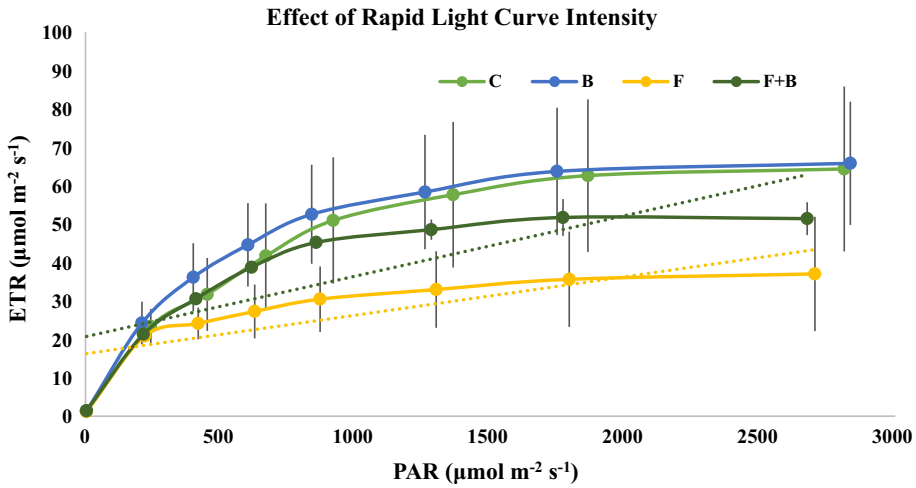

Fig. 7 ETR as a function of actinic irradiance. Rapid light curves of control (*C*), flooded (*F*), bacteria-treated (*B*) and flooded + bacteria-treated (*F* + *B*) *S. indicum* plant. Mean ETR are plotted with irradiance (µmol m^{-2} s^{-1})

in waterlogged condition. The increase in *Fo* value revealed the stress on plants and is also known as "increased minimal fluorescence." The variation in the quantum yield of PS-II (Y-II) and maximum relative electron transport rate (ETRm) values manifested the adverse effect of flooding stress on sesame plant (Table 2).

In general, the results of *Fv/Fm* are used to describe the effects of environmental stress conditions. At day 0 following flooding, *Fv/Fm* values for all treatment groups were more than 0.8, while a small fluctuation was observed on the second day of waterlogged condition. The unstressed control and bacteria-treated plants presented maximum *Fv/Fm* values until the experiment end. However, flooded plants showed a significant decrease in *Fv/Fm* value after day 4 of flooding. On the other hand, flooded plants treated with ACC deaminase-producing *P. veronii* KJ exhibited a significant increase in *Fv/Fm* value as compared with untreated waterlogged plants (Fig. 8). These results indicate that ACC deaminase-producing bacteria contribute to the photochemical process of plants in flooding stress condition.

The results for *Fv/Fm* were measured every second day throughout the experiment and allowed us to appropriately investigate the changes over time and across different treatment conditions. The standard value (0.83) of *Fv/Fm* was determined by Bjoerkman and Demmig [25], while the control plants in our experiments showed close approximation to the standard value throughout the experiment. However, *Fv/Fm* value for flooded sesame plants gradually declined after day 4 and reached 0.73 on day 8 of the experiment. On the other hand, plants treated with *P. veronii* KJ showed higher values for *Fv/Fm* until the experiment end (Fig. 8).

Discussion

Several ACC deaminase-producing bacteria have been known to promote plant growth and mitigate the effects of different environmental stresses, including waterlogging stress [18, 26, 27]. The study of Honma and Shimomura [13] showed the deamination of 1-aminocyclopropane-1-carboxylate (ACPC) as a sole nitrogen source by bacterial (*Pseudomonas* spp.) ACC deaminase. Later, Glick et al. [28] suggested a model for the amelioration of abiotic stress damage caused by ACC deaminase-producing rhizobacteria. The study of Li et al. [29] concluded the presence of *acdS* gene for the synthesis of ACC deaminase

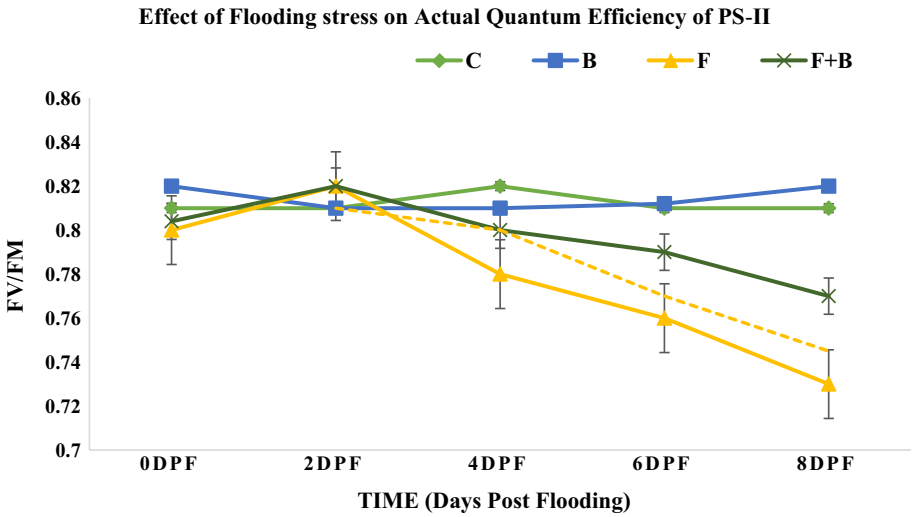

Fig. 8 The effect of flooding on actual quantum efficiency of PS-II. *Fv/Fm* of control (*C*), flooded (*F*), bacteria-treated (*B*), and flooded + bacteria-treated (*F* + *B*) *S. indicum* at different time points. Mean *Fv/Fm* values are plotted with time (post-flooding days)

and promotion of canola root elongation. In the present study, the phenotypic identification of *acdS* gene in the rhizobacterium *P. veronii* KJ paved the way for 16S rDNA sequencing, amplification, and sequencing of *acdS* gene, and quantification of ACC deaminase activity. Moreover, the alleviation of flooding stress-associated damage after bacterial treatment was confirmed through the changes in different growth-related variables such as root length, shoot length, fresh and dry biomass, concentration of chlorophyll, and chlorophyll fluorescence.

Pseudomonas spp. have been reported to produce ACC deaminase and reduce flooding stress-associated damage in crop plants [30–32]. We evaluated the ability to utilize ACC in DF minimal medium for the phenotypic identification of *acdS* gene (Fig. 1) and found that the test strain showed robust growth on DF minimal medium supplemented with the substrate ACC. This observation is consistent with the results of [32], wherein *Pseudomonas* isolate CPA123 was shown to produce ACC deaminase and utilize ACC as the sole nitrogen source on DF minimal media.

Drancourt et al. [33] confirmed *Pseudomonas*, *Agrobacterium*, and *Phyllobacterium* on the basis of 16S rDNA sequences. In agreement with this study, the direct amplification and sequencing of 16S rDNA sequences of *P. veronii* KJ revealed 99% homology to those of known *Pseudomonas* species (Fig. 2). According to Glick et al. [34] the mutants of *P. putida* lacking genes for ACC deaminase were unable to promote growth of canola seedlings. In our study, the precise confirmation of *acdS* gene in the characterized strain (*P. veronii* KJ) favored its use as a bioinoculant in waterlogged condition.

Prolonged water stress adversely affects different aspects of plant physiology, leading to a substantial decrease in the yield of crop plants, with the exception of rice [15, 35, 36]. In the present study, sesame plants were treated with ACC deaminase-producing *P. veronii* KJ and showed a significantly higher tolerance to waterlogging stress as compared with untreated flooded plants. In comparison with untreated stressed plants, flooded plants treated with ACC deaminase-producing bacteria showed a significant difference in root length, shoot length, fresh biomass, dry biomass, and chlorophyll content (Table 1, Fig. 5). This result is in line with that reported by Grichko and Glick [18]. These authors reported that the shoot height and fresh and dry shoot weight of tomato plants reduced after 9 days of flooding and that plants treated with ACC deaminase-producing bacteria showed significantly higher tolerance to flooding stress condition. It was suggested that the protection against flooding stress resulted in significant differences in the overall plant growth and leaf chlorophyll content. Recently, the study of Forghani et al. [37] revealed that upon initiation of stress condition the leaves of sweet sorghum increase the amount of total chlorophyll and carotenoid contents. Similarly, in our study, sesame plants initially showed a minor increase in the concentration of chlorophyll, which declined dramatically thereafter and more than 30% decrease was recorded in the chlorophyll content of waterlogged plants. On the contrary, plants treated with ACC deaminase-producing *P. veronii* KJ presented a significant increase in their chlorophyll content (Fig. 5). Grichko and Glick [18] also showed that leaf chlorophyll content decreased in flooded plants as compared with control plants and that the concentration of chlorophyll was significantly higher in flooded plants treated with ACC deaminase-producing bacteria as compared with untreated waterlogged plants. Thus, waterlogging stress may have a significant impact on plant photosynthesis and often leads to low photochemical efficiency in leaves [15]. In the present study, ETR of PS-II displayed a negative deviation after 8 days of waterlogging, and the reduced performance of ETR was more

obvious for the waterlogged plants than the control plants. On the other hand, bacteria-treated plants under waterlogged condition showed a minimum decrease in ETR value as compared to untreated plants, thereby highlighting their tolerance to waterlogging stress (Table 2). The decrease in ETR may serve as an indicator of electron transport chain deterioration in PS-II. This observation is consistent with the report of Smethurst and Shabala [15], wherein the adverse effect of waterlogging stress on photochemical process is revealed. Overall, our results indicate that waterlogged condition adversely affects different physiological aspects of sesame plant, while treatment with ACC deaminase-producing *P. veronii* KJ results in the alleviation of the damage and contributes to plant growth and development.

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