ABSTRACT
Salinity stress is one of the most serious environmental stress that inhibits plant growth. In this study, we adopted an environment friendly technique and screened different plant growth-promoting fungi for different plant growth promoting (PGP) traits and salinity stress. Among those isolates, Bipolaris sp. CSL-1 was selected based on plant growth-promoting characteristics, such as producing indole-3-acetic acid (IAA), gibberellins (GAs), organic acids and resistance to sodium chloride (NaCl) stress. Here, we found that inoculating soybean with isolate CSL-1 significantly increased shoot and root length, shoot and root fresh and dry weight and chlorophyll content under NaCl stress (200 mM). Endogenous abscisic acid (ABA) levels were significantly decreased, whereas salicylic acid (SA) levels were increased in CSL-1-inoculated plants under NaCl stress. NaCl-treated noninoculated plants showed higher levels of antioxidants and oxidative stress such as peroxidase, polyphenol oxidase (PPO), malondialdehyde (MDA) and superoxide anion (SOA). Furthermore, CSL-1 inoculation improved soybean resistance to NaCl stress, and there was a significant decrease in GmFDL19, GmNARK, and GmSIN1 expression levels. As a result, the fungal isolate CSL-1 mitigates the effect of salt stress and enhance soybean growth and might be used as a valuable ecofriendly microorganism resource in salt-affected areas.

Introduction
Salinization of soil with sodium chloride (NaCl) is one of the most environmental stresses that inhibits plant functions, causing reduction of crop yield and quality (Gupta et al. 2020). Currently, 20% of total cultivated land and 33% of irrigated agricultural land are affected by salinity stress worldwide. Studies have estimated that 50% of all arable land in the world will be affected by salinity stress in the year 2050, which will cause more than 12 billion USD annual losses due to reduced crop productivity (Shahbaz and Ashraf 2013; Shrivastava and Kumar 2015). Salinity is recognized as the major threat to environmental resources in several countries, affecting almost 1 billion hectares worldwide, which represents approximately 7% of the earth’s continental area (Gupta et al. 2020; Shrivastava and Kumar 2015). It is estimated that at global scale, 3 hectares of arable land become unproductive due to secondary salinization in every minute, therefore driving between 10 and 20 million hectares of irrigated land to zero productivity every years (Cuevas et al. 2019). It has been reported that soil salinity limits crop productivity by impairing root growth, nutrient uptake and affects various physiological, morphological, and biochemical processes that leads to reduction of crop biomass and productivity (Hamayun et al. 2010b; Khan et al. 2020a). Salinity stress causes morphological changes in all growth phases, including germination, seedling, vegetative, and maturity stages (Khan et al. 2019b; Tavakkoli et al. 2010). Biochemical changes include modulation of phytohormones such as increase in the stress hormone abscisic acid (ABA) and salicylic acid (SA) levels, changes in ion uptake levels (accumulation or removal of ions), activation of antioxidant enzymes, accumulation of reactive oxygen species (ROS), and alterations in photosynthetic pathways (Yoon et al. 2009). Salt induces toxic ions accumulation in plant cells through higher influx of Na+ ions and efflux of K+ ions (Kaya et al. 2020). Na+ and K+ homeostasis plays an essential role in the growth and development of crop plants (Rahneshan et al. 2018) and alters the metabolic process and ion mobilization system (Shrivastava and Kumar 2015). However, SA reduces oxidative stress by reducing Na+ uptake in the leaves, increasing the K+ content, and enhancing the leaf chlorophyll content and upregulation of antioxidant enzymes (Kaya et al. 2020).

Diverse strategies, such as development of salt resistant varieties, genetic engineering of halotolerant transgenic plants, leaching of salt from root zone, and chemical application are used to alleviate the toxic effects caused by salinity stress (Hamayun et al. 2017; Wang et al. 2003). Although using these approaches for sustainable management can ameliorate yield reduction under salinity stress, their implementation is often limited due to cost, being time-consuming, and availability of resources (Khan et al. 2021a). Development of efficient, low-cost, environment friendly
and easily adaptable methods for mitigating salinity stress is a major challenge for agricultural scientists (El-Esawi et al. 2018). In recent years, soil-borne microbes and plant-associated fungi have been extensively used due to their capacity to promote plant growth and being environmentally friendly for alleviating the toxic effects of salinity stress (Arnold et al. 2003; Barrow et al. 2008; Khan et al. 2011; Radhakrishnan et al. 2013). Microorganisms possess the ability to produce phytohormones, siderophores, and organic acids (Khan et al. 2011; Radhakrishnan et al. 2013). Various phytohormone-producing fungi have been reported to enhance plant growth under various environmental stresses (Bilal et al. 2018; Khan et al. 2011). Besides, some fungi, such as Trichoderma harzianum, are known to improve the quality of saline soil that can promote plant growth (Mbariki et al. 2017). Plant growth promoters, such as Bacillus siamentis, Bacillus firmus and Bacillus spp. are also used as growth promoters under stress condition, which enhance plant growth through regulation of the plant antioxidant defense system (Awan et al. 2020; El-Esawi et al. 2018).

Soybean growth is affected by various environmental factors, including salinity stress (El-Esawi et al. 2018; Khan et al. 2019a). Soybeans are rich sources of protein, oil, and flavonoids (El-Esawi et al. 2018; Hamayun et al. 2017; Rathod et al. 2011). However, exposure to salinity stress decreases the contents of all these compounds in soybean (Hamayun et al. 2017). Several soybean genes have been found to confer resistance to salinity stress (Li et al. 2019). For instance, GmFDL19 enhances resistance to salt stress by reducing the content of Na⁺ ions and malondialdehyde (MDA) and increasing the activity of several antioxidant enzymes and chlorophyll content in soybean (Li et al. 2017). Similarly, GmNARK expression is induced by ABA and NaCl treatment and sensitivity to salt stress (Cheng et al. 2018). Additionally, GmSN1 exhibited insensitivity to high salinity and resulted in higher stress activities of antioxidants, such as superoxide dismutase (SOD) and peroxidase (POD) (Li et al. 2019). The salinity stress inhibits K⁺ uptake, whereas GmAkt2 regulates K transport through electric cell signaling and membrane excitability. Furthermore, higher expression of GmAkt2 in soybean under salinity stress regulates potassium gradient that plays a vital role in osmotic adjustment and regulation of membrane potential and acts as a source of energy in plants. Therefore, in the present study, previously reported plant growth-promoting fungi from the Crop Physiology Laboratory, Kyungpook National University, South Korea, were grown and screened on potato dextrose agar (PDA) plates with different NaCl concentrations. Based on their high resistance to salt stress, the isolate CSL-1 was selected, and after inoculation, the growth attributes, endogenous phytohormones, antioxidant enzyme response, and expression of different salt-related genes under NaCl stress were observed in soybean plants.

**Material and methods**

**Isolation, screening, and identification**

Several plant growth-promoting endophytic fungi; Bipolaris sp (Lubna et al. 2019), Aspergillus fumigatus (Bilal et al. 2018), Cladosporium sp (Hamayun et al. 2010a), Cla. sphaerospermum (Hamayun et al. 2009), Fusarium proliferatum (Bilal et al. 2018) and Aspergillus niger (Lubna et al. 2018a) were screened for NaCl stress resistance using six concentrations of NaCl (0, 50, 100, 150, 200, and 250 mM), in 100 ml of sterilized Czapek media [glucose, 1%; peptone, 1%; MgSO₄·7H₂O, 0.05%; KCl, 0.5%; and FeSO₄·7H₂O, 0.001%; pH 7.3 ± 2] (Waqqas et al. 2014) and incubated in a shaking incubator at 30°C. Based on high tolerance during the screening experiments, the isolate Bipolaris sp. CSL-1 was selected for further experiments (S. Figure 1).

**IAA analysis of Bipolaris sp. CSL-1**

The isolate CSL-1 was grown in Czapek media for 7 days, centrifuged (500× g, 15 min), and analyzed for IAA and GAs content. IAA analysis was conducted according to the detailed method of Khan et al. (2021b). Briefly, 10 ml culture filtrate of the fungal isolate was centrifuged at 10,000× g at 4°C, and the supernatant was filtered through a 0.45-µm cellulose acetate filter, acidified through 2N NaOH/6N HCL to pH 2.8, and 50 ng ml⁻¹ [D₃]-IAA was added. 10 ml culture filtrate was then extracted three times with an equal amount of ethyl acetate. Afterwards, the organic layers were combined together and completely evaporated through a rotary evaporator in a water bath at 35°C. The dried extract was resuspended in 5 ml of 0.1 M acetic acid and then passed through Chromatography Column with Coarse Fritted Disc (10.5 300 mm; Pyrex; product no: 38450-10). The extract was eluted with 30% methanol (MeOH), 50% MeOH, and 100% MeOH. All the eluates were combined and then evaporated to complete drying under vacuum using a rotary evaporator (150 hPa). Methyl esters of the fractions were prepared by dissolving the residue in 1 ml methanol and adding 1.5 ml ethereal diazomethane. The excess diazomethane was removed under a stream of dry, oxygen-free nitrogen gas. Finally, the methylated sample was redissolved in ethyl acetate before being analyzed by gas chromatography–mass spectrometry (GC/MS) with selected ion monitoring (SIM; 6890N network GC system, and 5973 network mass-selective detector; Agilent Technologies, Santa Clara, CA, USA) (S. Table 1). The concentration of IAA was measured against the internal standard [D₃]-IAA. For quantification, the Lab-Base (ThermoQuest, Manchester, UK) data system software were used to monitor responses to ion of m/e 130 for IAA and 135 for [D₃]-IAA.

**GA analysis of Bipolaris sp. CSL-1**

The gibberellin content was evaluated according to the detailed method of Lubna et al. (2019). GAs were obtained from the pure culture of isolate CSL-1 grown in Czapek media for 7 days. The culture medium was separated through vacuum filtration. The obtained culture filtrate (CF) was then used for GA analysis. 50 ml of CF was partitioned with ethyl acetate, and the organic layer was vacuum dried. Next, 60% methanol (MeOH) was added, the pH was increased to 8.0 ± 0.3 by adding 2N NH₄OH, and deuterated GA internal standard was added. The methylated sample was dissolved in 60% MeOH, and passed through a Davisol C18 column (90–130 µm; Alltech, Deerfield, IL, USA); 5 g of C18 powder was used as the solid phase, and 60% MeOH was used as the mobile phase. The eluent was collected and concentrated at 40°C in vacuum. The sample was then dried onto Celite.
and loaded onto a SiO2 partitioning column to separate the GAs from the more polar impurities. GA extracts were eluted with 95:5 (v/v) ethyl acetate (EtOAc): hexane saturated with formic acid. This solution was dried at 40°C in vacuum, re-dissolved in EtOAc, and partitioned three times against 0.1 M phosphate buffer (pH 8.0). Next, 2N NaOH was required during the first partitioning to neutralize residual formic acid. Polyvinylpolypyrrolidone was added to the combined aqueous phases, and the resulting mixture was stirred for 1 h. The pH was reduced to 2.5 and the extract was partitioned three times against equal volumes of EtOAc. The combined EtOAc fraction was dried in vacuum (93 hPa), and the residue was dissolved in 100% MeOH. This solution was dried on a Savant Automatic Environmental Speedvac (AES 2000, Madrid, Spain). Extraction of GAs was further processed for fractionation in reversed-phase C18 high-performance liquid chromatography column (HPLC). For chromatography, a 3.9 × 300 mm Bondapak C18 Column (Waters corp., Milford, MA, USA) was used. The column was eluted at 1.5 ml/min with the following gradients and conditions: 0–5 min, isocratic 28% MeOH in 1% aqueous acetic acid; 5–35 min, linear gradient from 28% to 86% MeOH; 35–36 min, 86%–100% MeOH; and 36–40 min, isocratic 100% MeOH. A total of 48 fractions of 1.5 ml were obtained. For further analysis, these fractions vials were prepared for GC/MS gas chromatography–mass spectrometry with selected ion monitoring (SIM) (6890N network GC system, and 5973 network mass-selective detector; Agilent Technologies, Palo Alto, CA, USA). GA methyl ester was prepared with ethereal diazomethane. The sample was dried with nitrogen gas, re-dissolved in dichloromethane (CH2Cl2), and methylated one more time. The sample was dissolved in 30 µL pyridine and 30 µL N, O-bis (trimethylsilyl)–trifluoroacetamide with 1% trimethylchlorosilane (Pierce Chemical Co., Rockford, IL, USA), and silylated for 30 min at 65°C. This sample was then dried with nitrogen gas and solubilized in anhydrous dichloromethane. A sample of 1 µl for each GA was injected into a 30 m × 0.25 mm i.d., gas and solubilized in anhydrous dichloromethane. A sample 30 min at 65°C. This sample was then dried with nitrogen gas, re-dissolved in dichloromethane (CH2Cl2), and methylated one more time. The sample was dissolved in 30 µL pyridine and 30 µL N, O-bis (trimethylsilyl)–trifluoroacetamide with 1% trimethylchlorosilane (Pierce Chemical Co., Rockford, IL, USA), and silylated for 30 min at 65°C. This sample was then dried with nitrogen gas and solubilized in anhydrous dichloromethane. A sample of 1 µl for each GA was injected into a 30 m × 0.25 mm i.d.,

**Organic acid analysis of Bipolaris sp. CSL-1**

For organic acid analysis, the culture filtrate of the isolate CSL-1 was filtered through 0.22-µm Millipore filter (ADVANTE; DSMIC-25CS), and 20 µl of each sample was injected into a HPLC: Shimadzu Waters Co., Model 600E, included refractive index detector, RI model RID-140A, and loaded onto a SiO2 partitioning column to separate the GAs from the more polar impurities. GA extracts were eluted with 95:5 (v/v) ethyl acetate (EtOAc): hexane saturated with formic acid. This solution was dried at 40°C in vacuum, re-dissolved in EtOAc, and partitioned three times against 0.1 M phosphate buffer (pH 8.0). Next, 2N NaOH was required during the first partitioning to neutralize residual formic acid. Polyvinylpolypyrrolidone was added to the combined aqueous phases, and the resulting mixture was stirred for 1 h. The pH was reduced to 2.5 and the extract was partitioned three times against equal volumes of EtOAc. The combined EtOAc fraction was dried in vacuum (93 hPa), and the residue was dissolved in 100% MeOH. This solution was dried on a Savant Automatic Environmental Speedvac (AES 2000, Madrid, Spain). Extraction of GAs was further processed for fractionation in reversed-phase C18 high-performance liquid chromatography column (HPLC). For chromatography, a 3.9 × 300 mm Bondapak C18 Column (Waters corp., Milford, MA, USA) was used. The column was eluted at 1.5 ml/min with the following gradients and conditions: 0–5 min, isocratic 28% MeOH in 1% aqueous acetic acid; 5–35 min, linear gradient from 28% to 86% MeOH; 35–36 min, 86%–100% MeOH; and 36–40 min, isocratic 100% MeOH. A total of 48 fractions of 1.5 ml were obtained. For further analysis, these fractions vials were prepared for GC/MS gas chromatography–mass spectrometry with selected ion monitoring (SIM) (6890N network GC system, and 5973 network mass-selective detector; Agilent Technologies, Palo Alto, CA, USA). GA methyl ester was prepared with ethereal diazomethane. The sample was dried with nitrogen gas, re-dissolved in dichloromethane (CH2Cl2), and methylated one more time. The sample was dissolved in 30 µL pyridine and 30 µL N, O-bis (trimethylsilyl)–trifluoroacetamide with 1% trimethylchlorosilane (Pierce Chemical Co., Rockford, IL, USA), and silylated for 30 min at 65°C. This sample was then dried with nitrogen gas and solubilized in anhydrous dichloromethane. A sample of 1 µl for each GA was injected into a 30 m × 0.25 mm i.d., 0.25-µm film thickness DB-1 capillary column (J & W Scientific Co., Folsom, CA, USA). The GC oven temperature was programmed as follows: 1-min hold at 60°C and then increased to 285°C at 1 to 200°C, followed by 5°C min −1 to 285°C (S. Table 2). Helium carrier gas was maintained at a head pressure of 30 kPa. The GC was directly interfaced to a mass-selective detector with an interface and a source voltage of 70 eV and a dwell time of 100 ms. First trial (full-scan mode) and three major ions of the supplemented [2H2]GA were monitored simultaneously. Retention times of the GA and hydrocarbon standards were determined using the described chromatographic conditions and the Kovat Retention Indices (KRI) calculated (Table S3). The GAs were quantified against the deuterated internal standards using the most intense of the three selected ions for each GA (Table S3). The data were calculated in ng/ml.

**Plant growth and Bipolaris sp. CSL-1 role under salinity stress**

Soybean seeds variety pungssamnamul were collected from the Soybean Genetic Resource Center (Kyungpook National University Daegu, Republic of Korea). First, the seeds were surface-sterilized using 2.5% sodium hypochlorite for 15 min and washed three times with autoclaved double-distilled water. These sterilized seeds were placed in plastic trays filled with a horticulture substrate containing cocom peat (45%–50%), perlite (35%–40%), peat moss (10%–15%), and zeolite (6%–8%), along with NO3 (~0.205 mg/g), K (0.1 mg/g), NH4 (~0.09 mg/g), and PO4 (~0.35 mg/g) (Shinsung Mineral Co., Ltd., Goesan, Korea) (Asaf et al. 2017b). The seedlings were grown in a growth chamber at 28°C ± 0.5°C for 16 h and at 25°C for 8 h, with 55%–65% relative humidity and 200 µmol m−2 s−1 light intensity under long-day conditions (16 h of day time and 8 h of night time) for 2 weeks. After 2 weeks of germination, equal-sized seedlings were transferred to pots (440 × 270 × 195 mm) filled with the same horticulture substrate used in the germination trays and were grown in a growth chamber. The experimental design included (a) control-well watered, (b) fungal-treated CSL-1, (c) 200 mM NaCl stress, and (d) 200 mM NaCl stress with the isolate CSL-1. A suspension of 1 × 10^6 conidia ml−1 was used in the experiments as reported previously (Tefera and Pringle 2009). To test the plant-protection activity of CSL-1 under salinity stress, a 5 ml conidial suspension (1 × 10^6 conidia ml−1) was applied around the root zone of each seedling four times after every four days up to 16 days. The control plants were inoculated with 5 ml sterile distilled water followed the same method. Seedlings in all treatments were watered as needed. To avoid conidial runoff from the treated leaves to the stem and soil, watering device was carefully directed to the surface of the pot. After 16 days, 10 ml of salt solution (200 mM NaCl) was applied to each seedling on daily basis for 7 days.

**Morphological analyses**

The growth attributes (root and shoot length) and biomass (root/shoot fresh and dry weight) was determined after two weeks of salt treatment. The remaining fresh plants were harvested in liquid nitrogen and stored at −80°C for further biochemical analyses. Before the harvest, the chlorophyll contents were measured using the SPAD chlorophyll meter (SPAD-502 Minolta, Tokyo, Japan).

**Quantification of endogenous abscisic acid (ABA)**

For endogenous ABA analysis, 0.3 g of powdered shoot was treated with 30 ml of extraction solution (95% isopropanol and 5% glacial acetic acid) and 10 ng of ABA standard [(±)-3,5,5,7,7,7-d6] obtained from the National Research
Council of Canada-Plant Biotechnology Institute. The filtrate was concentrated in a rotary evaporator. The residue was dissolved in 4 ml of 1 N sodium hydroxide solution and then washed three times with 3 ml of methylene chloride to remove lipophilic materials. The aqueous phase, brought to a pH value of approximately 3.5 using 6 N hydrochloric acid, was partitioned three times into ethyl acetate (EtOAc). The EtOAc extracts were then combined and evaporated. The dried residue was dissolved in a phosphate buffer (pH 8.0) and then run through a polyvinylpolypyrrolidone (PVPP) column (Corning Pyrex Borosilicate Glass Chromatography Column with Coarse Fritted Disc, Inner Diameter 10.5 mm × Height 300 mm, 25 ml Capacity). The pH value of the phosphate buffer was adjusted to 3.5 using 6 N HCl, and the sample was partitioned three times into EtOAc. The EtOAc extracts were combined again and evaporated. The residue was dissolved in dichloromethane (CH2Cl2) and passed through a 6 cc silica cartridge (Sep-Pak; Waters Associates, Milford, Massachusetts, USA) prewashed with 10 ml of diethyl ether:methanol (3:2, v/v) and 10 ml of trifluoracetic acid without pyrogallol (B) and control (C) was represented as enzyme unit (EU) nmol/g.

Superoxide dismutase (SOD) level was measured according to the detailed method of Sahile et al. (2021). Leaf tissues were ground with 1.3 ml of buffer containing 50 mM Tris-HCl and 10 mM EDTA (pH 8.0). The extracted sample (100 µl) and 100 µl pyrogallol (7.2 mM) were mixed and allowed to stand at 25°C for 10 min. The reaction was stopped by adding 50 µl of 1N HCl, and then it was measured at 420 nm. The percentage of SOD activity was calculated using the following formula: SOD activity (%) = \[1 - (A - (A - B)/C)\] × 100. The extraction sample added with (A) or without pyrogallol (B) and control (C) was represented as buffer solution added with pyrogallol. The SOD activity was expressed as enzyme unit (EU) nmol/g.

For lipid peroxidation level, 0.1 g of fresh leaves was extracted in 3 ml of 1% TCA, homogenized in an ice-cold mortar and pestle, and centrifuged (10,000 rpm, 15 min at 4°C). Then, 2 ml of supernatant and 4 ml of 0.5% TBA were heated at 95°C for 30 min and cooled immediately in an ice bath. The absorbance of the sample was measured at 532 and 600 nm in a spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Vantaa, Finland) using the MDA equation = 1000[(ABS532−ABS600 nm)/155] (Chaoui et al.1997). The lipid peroxidation levels were then calculated against a malondialdehyde standard and the results expressed as mmoles MDA formed per gram of leaf tissue.

Quantification of endogenous salicylic acid (SA)

For SA analysis, 0.2 g of freeze-dried fine powder was sequentially extracted with 2 ml of 90% and 100% methanol by centrifuging for 15 min at 10,000×g. The combined methanol extract was evaporated in vacuum centrifuged and vacuum-dried. The dried pellets of the sample were resuspended in 3 ml of 5% trichloroacetic acid after centrifugation, and the supernatant was partitioned using ethyl acetate, cyclopentane, and isopropanol (ratio of 49.5:49.5:1, v/v/v). The top organic layer containing free SA was transferred to 4-ml tubes and dried with nitrogen gas. The dried SA was again suspended in 1 ml of 70% methanol. Next, 25 µl of the filtered sample was subjected to HPLC in a C18 reverse-phase HPLC column (HP hypersil ODS, particle size 5 µm, pore size 120 Å Waters; size 3.9 × 300 mm) at a flow rate of 1.0 ml/min (Jan et al. 2020b) (S. Table 6). SA was detected using a Shimadzu fluorescence detector (Shimadzu RF-10AXL), with excitation and emission monitored at 305 and 365 nm, respectively. The quantity of SA was calculated according to the peak value for authentic standards.

Quantification of total protein

For protein analysis, fresh leaf tissues (250 mg) were crushed in an ice-cold mortar and pestle and then added 2 ml solution of 50 mM phosphate-buffered saline (pH 7.0), 0.1% polyvinylpyrrolidone (PVP), 3 mM MgCl2, and 1 mM ethylene diamine (EDTA). The homogenate was centrifuged at 10,000×g for 10 min at 4°C. The resulting supernatant was immediately collected and used for protein quantification (10 µl plant extract and 190 µl Bradford reagent). The protein content was measured at 595 nm using the Bradford (Bradford 1976) method with BSA as the standard.
RNA extraction, cDNA synthesis, and qRT-PCR analysis

RNA extraction was performed according to the protocol described by Chan et al. (2004). Briefly, total RNA was extracted using 1 ml of Trizol® reagent (Invitrogen, USA) from 100 mg of crushed leaves of soybean, incubated at room temperature (10 min), and then centrifuged (12,000 g; 10 min; 4°C). The supernatant was transferred to new tubes (1.5 ml), and then 200 µl of chloroform was added, vortexed vigorously (15 s), set on ice (3 min), and then centrifuged (12,000 g; 15 min; 4°C). The upper layer (300–400 µl) was transferred to a new tube, and 500 µl each of iso-propanol and 1.2 M NaCl/0.8M Na-Citrate was added, kept for 10 min at room temperature, and then centrifuged (12,000 g; 10 min; 4°C). The pellets were washed with 1 ml of 75% EtOH and centrifuged again (7500×g; 5 min at room temperature), the supernatant was discarded, and the pellet was resuspended in 40 µl of DEPC water.

For cDNA synthesis, the qPCRBIO cDNA Synthesis Kit from PCRBIOSYSTEMS was used, and 5 µg of total RNA was used in reverse transcription (Jan et al. 2021). Briefly, 1 µl of cDNA was used for qRT-PCR performed using the qPCRBIO SYBER Green Kit from PCRBIOSYSTEMS according to the detailed method of Jan et al. (2020a). Actin was used as a reporter gene (S. Table 7).

The Eco Real-Time (Illumina, Singapore) machine was used for qRT-PCR conducted using 20 µl (10 µl SYBR green, 7 µl ddH2O, 1 µl template DNA, and 1 µl primer) of reaction volume. A total sample volume of 20 µl was subjected to the following conditions: initial denaturing at 94°C (5 min), 40 cycles of denaturation at 94°C (30 s), annealing at 58°C (30 s), extension at 72°C (1 min), and final extension at 72°C (5 min).

Determination of Na+ and K+ uptake in plant

The Na+ and K+ content in the shoot of fungal-inoculated and -noninoculated normal and NaCl-stress-exposed soybean plants was investigated according to the detailed method of Kang et al. (2021) and Kubi et al. (2021). Briefly, the freeze-dried sample (0.3 g) was suspended in 5 ml of HNO3 and digested with 3 ml H2O2. The obtained solvent was quantified by inductively coupled plasma mass spectrometry (ICP-MS; Optima 7900DV, Perkin-Elmer, USA).

Statistical analysis

Results were statistically evaluated by the analysis of variance (ANOVA) using the SAS 9.4 software. We performed experiment with three replicates, each replicate has 10 plants (total number of plants, n = 30/treatment). Then we averaged the results of these 10 plants to get one value /replicate and then used these 3 values /treatments (n = 3) to performed statistical analysis. Error bars indicate the SD between biological repeats (n = 3). All values are expressed as mean ± standard deviation. Mean values were analyzed using Duncan’s multiple range tests with significant difference among treatments by ANOVA.

Results

Isolation and screening for salt tolerance and identification

The previously isolated, plant growth-promoting endophytic fungi Bipolaris sp. CSL-1 was obtained from fungal stock at the Crop Physiology Laboratory, Department of Applied Biosciences, Kyungpook National University, Korea, and screened for NaCl stress tolerance. The screening results showed that the isolate CSL-1 can grow in Czapek media with up to 200 mM NaCl. Therefore, the isolate CSL-1 was selected for future research.

In vitro quantification of IAA, GAs, and organic acids in the culture filtrate of the isolate CSL-1

GC/MS and HPLC were used to quantify IAA, GAs, and organic acid compound in the culture filtrate of the isolate CSL-1. The results of IAA analysis revealed that the isolate CSL-1 produces IAA (0.62 ± 0.02 ng/ml: Figure 1A). However, different levels of bioactive GAs were detected in the culture broth of CSL-1 (Figure 1B and S. Fig. 2A-C). Likewise, in the culture filtrate (CF) of the isolate CSL-1, organic acids, such as citric acid, quinic acid, and succinic acid were found (Figure 1C).

CSL-1 regulates soybean growth under salinity stress

Salinity stress had adverse effects on the growth attributes of soybean plants. However, the isolate CSL-1 significantly enhanced the salinity stress tolerance by regulating the plant growth, plant biomass, and other biochemical attributes (Figure 2; Table 1). Under salinity stress, there were decreases in shoot length (25%), root length (46%), shoot fresh and dry weight (25% and 37%, respectively), and root fresh and dry weight (49% and 51%, respectively) at 200 mM of NaCl stress compared with those in control plants. However, the isolate CSL-1 induced significant increases in shoot length (16%), root length (37%), shoot fresh and dry weight (19% and 25%, respectively), and root fresh and dry weight (47% and 51%, respectively) in salinity-stressed plants compared with those observed at 200 mM NaCl-treated plants (Table 1). The chlorophyll contents were also increased22% in CSL-1-inoculated soybean plants compared with that in control plants under normal conditions (Table 1). However, under NaCl stress, the plants showed a decrease in chlorophyll content (25%) (Table 1). Nevertheless, inoculation with the halotolerant isolate CSL-1 mitigated the effects of NaCl stress and increased the chlorophyll content (24%) (Table 1).

Effect of CSL-1 on plant endogenous phytohormones

The level of abscisic acid (ABA) in soybean plants under salt stress increased by 160% compared with that in control soybean plants (only water treatment) (Figure 3A). However, when CSL-1-inoculated soybean plants were compared with NaCl stress-exposed plants (200 mM), the increased level of ABA was limited to 133% (Figure 3A). In contrast to endogenous ABA levels, there was a 2% increase in endogenous SA levels in CSL-1 inoculated plants compared with control plants under normal conditions. The salinity-stressed soybean plants showed a 28.56% decrease in endogenous SA levels compared with control soybean plants (only water). Compared with NaCl-stressed soybean plants, plants inoculated with CSL-1 showed improved NaCl stress tolerance and higher endogenous SA levels (22.3%) were observed (Figure 3B).
Antioxidant and oxidative stress quantification in soybean plants under salinity stress

The changes in different antioxidant and reactive oxygen species production levels were investigated in soybean plants treated with NaCl stress with the inoculation of CSL-1. The MDA content was evaluated to assess the extent of lipid peroxidation (LPO). Results showed higher MDA levels (2-fold) in soybean plants treated with NaCl stress (200 mM) than in plants with CSL-1 inoculation (0.5-fold) (Figure 4A). Also, the SOA results revealed an increase with NaCl treatment (29%). However, the production of SOA was significantly inhibited in CSL-1-inoculated soybean plants (38%–91%) (Figure 4B). A similar trend was observed in POD and PPO levels, which were lower in salinity-stressed soybean plants inoculated with CSL-1 (Figure 4C&D). To further elucidate the mitigation of salinity stress, the GSH and LPO content in soybean plants was examined, which revealed a significant enhancement in GSH (61%) and LPO (116%) content under NaCl levels (200 mM). However, a decrease in GSH (28%) and LPO (37%) content were observed in CSL-1-inoculated plants compared with NaCl stressed plants (Figure 4E). Furthermore, the total protein content showed a significant decrease (31%) in plants exposed to salinity stress compared with that in control plants. However, the total protein content was increased in soybean plants inoculated with the halotolerant isolate CSL-1 (18%) compared with that in control stressed plants (Figure 4F).

Role of CSL-1 in ion uptake during salinity stress

The inductively coupled mass spectrometry (ICP) analysis of Na⁺ and K⁺ content showed that soybean plants treated with NaCl (200 mM) had an increased Na⁺ content (11-fold) (Figure 5A). However, in CSL-1-inoculated plants, there was a significant decrease in the Na⁺ content (7-fold) (Figure 5A). Compared with Na⁺, the K⁺ content was significantly decreased under salinity stress (15%) compared with that in control plants (Figure 5B). In contrast, the K⁺ uptake content was increased (7%) in soybean plants inoculated with the isolate CSL-1 compared with that in control stressed plants (Figure 5B).
Gene expression during salinity stress

GmFDL19 was highly expressed (8.6-fold) in soybean plants exposed to NaCl stress (200 mM) (Figure 6A). However, CSL-1 inoculation improved the adaptability of soybean plants against NaCl stress, with a significant decrease in GmFDL19 expression (5-fold) in plants exposed to NaCl stress (200 mM) (Figure 6A). In contrast, the expression levels of GmNARK and GmSIN1 were significantly increased in soybean plants exposed to NaCl stress (7- and 6-fold, respectively) (Figure 6B&C). However, CSL-1 inoculation enhanced the resistance of soybean plants to NaCl stress and reduced the expression of GmNARK and GmSIN1 (4- and 3-fold, respectively) in soybean plants exposed to NaCl stress (200 mM) (Figure 6B&C). In contrast to the expression of GmFDL19, GmNARK, and GmSIN1, GmAKT2 expression was found to be decreased (24%) in plants exposed to salinity stress compared with that in control plants. However, inoculation with the halotolerant isolate CSL-1 resulted in a higher expression of GmAKT2 (15%) in stressed soybean plants compared with that in control stressed plants (Figure 6D).

Discussion

Salinity stress has a significant negative impact on the productivity of crops (Abdel Latef and Chaoxing 2011). Salinity stress affects the morphological, biochemical, physiological, and molecular processes of plants, such as seed germination, growth, and nutrient uptake (Abdel Latef and Chaoxing 2011). In the present study, exposure to NaCl stress inhibited the growth, root/shoot length, and biomass (fresh/dry weight) of the soybean plant (Figure 2; Table 1). The beneficial effects of the isolate CSL-1 on soybean growth were observed in terms of root/shoot length and biomass (fresh/dry weight) (Figure 2; Table 1). These results are consistent with previous studies (Abdel Latef and Chaoxing 2011; Kaya et al. 2009; Molina-Montenegro et al. 2020) that reported that the NaCl-tolerant Penicillium brevicompactum, P. chrysogenum, and mycorrhizal fungi augmented salinity stress and enhanced plant growth attributes in tomato, lettuce, and pepper. The chlorophyll content plays a vital role in photosynthesis and, to some extent, indicates plant tolerance to salinity stress (Takai et al. 2010). Under salinity stress, there was a decrease in chlorophyll content in the soybean plant (Table 1). The reduction in chlorophyll content under salinity stress might be due to a decrease in K+ absorption (Dai et al. 2009). Higher Na+ uptake has an antagonistic effect on K+ absorption and suppresses specific enzymes that are responsible for the synthesis of chlorophyll (Dai et al. 2009; Murkute and Singh 2006). However, the increase in chlorophyll content in CSL-1-inoculated plants suggests that chlorophyll synthesis is less affected by salinity stress. Higher chlorophyll contents has been reported previously in zucchini, pepper, and tomato plants under saline stress treated with mycorrhizal fungi (Colla et al. 2008; Hajiboland et al. 2010; Kaya et al. 2009). Higher Na content in the soil inhibits the uptake of K and results in nutrient imbalance (Parida and Das 2005). K+ plays a vital role in stomatal conductance and enzyme activation (Khalil et al. 2011). In the present study, salinity stress significantly decreased the K+ content (Figure 5B). However, our results support previous findings (Evelin et al. 2012; Giri et al. 2007), which showed that the inoculation of mycorrhizal fungi enhanced K+ uptake under salinity stress. The increase in Na+ content has also been previously reported in mycorrhiza-inoculated plants (Evelin et al. 2012; Lu et al. 2014; Pollastri et al. 2018; Yanwei et al. 2014), which supports our results that soybean plants inoculated with CSL-1 exhibited decreased Na+ uptake and enhanced plant growth (Figure 5A).

Table 1. Effect of the isolate CSL-1 on the growth attributes and chlorophyll content of soybean plants under normal and salinity stress conditions. SL (shoot length), RL (root length), SFW (shoot fresh weight), RFW (root fresh weight), SDW (shoot dry weight), RDW (root dry weight), and CC (chlorophyll content). Each value represents mean ± SD of three replicates (n = 3). Values with different letters in columns are significantly different from each other as evaluated by DMRT analysis.

| Treatment | SL (cm) | RL (cm) | SFW (g) | RFW (g) | SDW (g) | RDW (g) | CC (SPAD) |
|-----------|---------|---------|---------|---------|---------|---------|-----------|
| Control   | 19.66 ± 0.57b | 14.66 ± 0.54b | 15.33 ± 0.41b | 11.06 ± 0.51b | 4.60 ± 0.20b | 1.10 ± 0.10b | 27.47 ± 1.52b |
| CSL-1     | 24.33 ± 0.55a | 19.07 ± 1.00a | 17.16 ± 1.04a | 14.10 ± 1.01a | 6.28 ± 0.30a | 1.30 ± 0.11a | 32.33 ± 2.30a |
| 200 mM NaCl | 15.01 ± 2.64c | 7.67 ± 0.57d | 10.80 ± 0.72d | 5.68 ± 0.25d | 2.75 ± 0.31d | 0.54 ± 0.02d | 20.33 ± 1.52c |
| 20 mM + CSL-1 | 20.00 ± 1.00b | 11.01 ± 1.14c | 13.86 ± 0.23c | 8.47 ± 0.50c | 3.35 ± 0.25c | 0.79 ± 0.08c | 24.83 ± 2.08b |

Figure 3. Endogenous abscisic acid (ABA) and salicylic acid (SA) level in soybean plants inoculated with CSL-1 under normal and NaCl stress conditions. Each data point is the mean of at least three replicates. Error bars represent standard errors. The bars presented with different letters are significantly different from each other as evaluated by DMRT analysis.
Phytohormone-producing plant growth-promoting endophytic fungi produce different plant hormones, e.g. gibberellins, indole-3-acetic acid, and organic compounds that help the plant tolerate or avoid abiotic stresses, including salinity stress (Ikram et al. 2018; Lubna et al. 2018a). Previous studies (Hamayun et al. 2010a) and (Waqas et al. 2012) have reported that the GA-producing endophytic fungi *Phoma herbarum* and *Penicillium* sp. mitigate salinity stress and enhance plant growth in soybean and cucumber. In culture filtrate of isolate CSL-1, different bioactive GAs were detected (Figure 1B). Among these GAs types, the amount of GA1 and GA4 were found higher than others GAs. Hasan (2002) found similar results in fungal species that secrete GAs, including *Aspergillus niger*, *Fusarium oxysporum*, *Perylophilum corylophilum*, *P. cyclopium*, *P. funiculosum*, and *Agrostis stolonifera*. Furthermore, the most important trait of plant growth-promoting fungi (PGPF) has been characterized as the synthesis of GAs by endophytic fungi (Zhang et al. 2016). Examples of important fungal endophytes having the ability to produce GAs are *Gibberella fujikuroi* (Robinson et al. 2001), *Pe. citrinum* (Jung et al. 2002), *Porostereum spadiceum*, *Neurospora crassa* (Jung et al. 2002), *Aspergillus flavus* CHS1 (Lubna et al. 2018b), *Aspergillus fumigatus* and *Fusarium proliferatum* (Bilal et al. 2018). Endophytes that produce GA are especially beneficial in agricultural land that is nutrient-poor and subjected to a variety of stressors. Various studies show that plants with endophytes are generally healthier, which could be owing to the production of phytostimulatory hormones such IAA and GAs (Bömke et al. 2008). IAA is a key phytohormone that plays an important role in plant growth and tolerance to different abiotic stresses by regulating several developmental and physiological processes (Ikram et al. 2018; Ismail et al. 2018). This is also consistent with the finding of our study that the isolate CSL-1 produced IAA and enhanced the growth, biomass, and tolerance of soybean plants to salinity stress upon inoculation (Figure 1A). Recently, Yousaf et al. (2021) reported that elevated IAA levels produced by CSL-1 colonization downregulated brassinosteroids (BRs) synthesis, compromising host cell virulence resistance and switching endophytic CSL-1 to pathogenic fungus (Yousaf et al. 2021). However, here we found that CSL-1 is an endophytic fungus that stimulates plant growth (Figure 1A). These findings might be attributed to salt stress, as it was previously shown that NaCl stress lowered IAA levels in plants significantly (Nilsen and David 1996; Wang et al. 2001). Furthermore, most fungal strains behaved as weak parasites, but their impacts on plant growth were strain and plant-dependent, as reported by many researchers, and could be explained partly by their morphological, physiological, and ecological characteristics (Kia et al. 2018). Other important osmolytes in plants are organic acids that are found in plant vacuoles and regulate crucial functions in abiotic stress tolerance, including salinity stress (Gupta et al. 2020). Previously, it was reported that fungal endophytes release organic compounds that assimilate plant growth under saline conditions (Yang et al. 2015; Zhao et al. 2014). The isolate CSL-1 used in our study produced different types of organic acids that might be helpful to soybean tolerate NaCl stress (Figure 1C). In plants, organic acids like citric acid and malic acid are key osmolytes. They are present in plant vacuoles, and the modulation of their metabolism is critical for salt stress tolerance (Guo et al. 2010). Fungal endophytes have been shown to influence...
the amounts and profile of organic acids in plants by inducing the release of organic compounds by the roots (Yang et al. 2015). A report shows that, endophytes can help their hosts get Fe by secreting organic acids that chelate and solubilize iron in the soil (Khan et al. 2006). It has been shown that the nutrient availability in the soil, by the release of organic acid from endophytes resulted in ferric solubilization to form organic ferric salt that can be assimilated directly by plants under saline condition (Zhao et al. 2014).

Under salinity stress, higher ROS levels are generated in different compartments of the plant cell that disrupt the normal metabolism of plants (Jabeen et al. 2020; Jithesh et al. 2006; Muchate et al. 2016). ROS are typically generated by the transfer of one, two, or three electrons to molecular oxygen to form superoxide (O$_2^−$), hydrogen peroxide (H$_2$O$_2$), or hydroxyl radical (OH) (Ahmad et al. 2016; Rasel et al. 2020). However, plants accelerate the activation of a proficient antioxidant system to abate the oxidative impairment caused by salinity stress (Awan et al. 2020; Rasel et al. 2020). Higher LPO contents in soybean plants under salinity stress suggest enhancement of lipid peroxidation and protein oxidation, which is consistent with previous findings (Navarro et al. 2013; Talaat and Shawky 2014). For mitigation of salinity stress and ROS generation, plants activate their antioxidant defense systems, such as POD and other nonenzymatic antioxidants (PPO and total protein) (Hasanuzzaman et al. 2020; Jabeen et al. 2020; Kim et al. 2020). SOD mediates the detoxification of superoxide radicals and prevents stress-induced cellular damage (Ighodaro and Akinloye 2018). Some nonenzymatic antioxidants, such as total protein, glutathione, and phenolic compounds, in plants are involved in the internal detoxification of NaCl-induced toxicity (AbdElgawad et al. 2016). Higher total protein, PPO, and GSH levels were observed in CSL-1-inoculated soybean plants under NaCl stress (Figure 4). Our study suggests that the total protein content was decreased under NaCl stress, whereas inoculation of the halotolerant isolate CSL-1 increased the TP content in salinity-stressed soybean plants (Figure 4F). These antioxidants play an essential role in salinity tolerance and better growth, which were reflected in various morphological and physiological parameters of soybean plant growth under NaCl stress. Several authors have earlier reported higher enzymatic antioxidant activities in plants inoculated with endophytic fungi than in noninoculated plants (Hajiboland et al. 2010; Lu et al. 2014; Yanwei et al. 2014). To cope with the damage caused by salinity stress, plants regulate their hormone synthesis (Ren et al. 2018; Van Ha et al. 2014). Abscisic acids is involved in cellular responses to salinity-induced toxicity (Van Ha et al. 2014). It has been shown that salinity stress increases the ABA content in plants (Saneoka et al. 2001; Van Ha et al. 2014). The plant–microbe interaction has been reported to mitigate the adverse effects of abiotic stress in plants by reducing ABA levels (Jan et al. 2019). The results of our study showed that the inoculation of NaCl-tolerant CSL-1 enhanced plant growth parameters and mitigated NaCl stress through the reduction of ABA accumulation (Figure 3A). Furthermore, SA is a defense-signaling phytohormone that acts as a scavenger of ROS and protects plant from oxidative stress (Kaya et al. 2020; Khan et al. 2021b). Previous research has shown that the exogenous application of SA had a protective role under salinity stress by improving K$^+$ uptake, reducing Na$^+$ content, and playing a key role in triggering antioxidant defense enzymes against ROS-induced damage (Arif et al. 2020; Kaya et al. 2020). Our results showed higher SA content in soybean plants inoculated with CSL-1. These findings confirm the previous reports of Kang et al. (2014) and who showed that microbial inoculation enhances SA content in plants under abiotic stress.

_Glycine max_ is a protein- and oil-rich crop (Rathod et al. 2011). However, salinity adversely affects its growth and development (Sheteiwy et al. 2021). Whenever salinity is a prominent soil problem, there is also a natural solution for it in the form of salt-stress-tolerant fungi that exhibit plant indigenous salt stress (Sheteiwy et al. 2021). To validate the function of the fungus _Bipolaris_ sp. in response to salt stress, we evaluated the expression of the salt-induced _G. max_ nodule autoregulation receptor kinase (GmNARK) gene that is induced by salt stress. The results showed that GmNARK expression level was higher in NaCl-treated plants, whereas NaCl-treated plants inoculated with the fungus exhibited reduced expression level of this gene as shown in Figure 6B. This result indicates that _Bipolaris_ sp. is significantly involved in the mitigation of salt stress. Previous research shows that NARK is also involved in JA signaling and alters the plant defense system (Kinkema and Gresshoff 2008). Cheng et al. (2018) reported that GmNARK overexpression in _Arabidopsis_ enhanced ABA levels and NaCl
tolerance, indicating that this gene is an important candidate for salt tolerance. They also predicted that similar to calcineurin B-like protein (CBL10), NARK may be involved in fast signal transduction in the cell under salinity. CBL10 functions as a calcium sensor in response to salt stress. Our study showed that the \textit{GmFDL19} expression pattern was identical to that of \textit{GmNARK} (Figure 6A). The expression level was reduced in NaCl-treated fungus-inoculated plants compared with that in pure NaCl-treated plants, indicating that \textit{Bipolaris} sp. is functionally involved in salt tolerance. We further expected that \textit{Bipolaris} sp. is involved in NaCl uptake that protects the plant from salt stress by preventing the direct contact of NaCl to plant cells. \textit{GmFDL19} is also a salt-induced gene and is expressed during salt stress by reducing the uptake of Na\(^+\) and enhancing the expression of stress- and ABA-responsible genes (Li et al. 2017). The over-expression of \textit{GmFDL19} in soybean plants positively regulated multiple stresses through the activation of the antioxidant machinery (Li et al. 2017). \textit{GmAKT2} is a K\(^+\) transporter gene involved in plant stress tolerance (Zhou et al. 2014). Potassium is a key component of the cell that plays a vital role in growth and development. It is been reported that K\(^+\) alters plant metabolic and hormonal pathways, which further enhances plant tolerance to multiple stresses (Amtmann et al. 2008). Plants regulate the manifestation and activities of numerous membrane transporters, such as high-affinity K transporters, that facilitate the transportation of cations across the membrane to maintain the Na/K ratio under salinity stress (Rasel et al. 2020). In contrast to \textit{GmNARK} and \textit{GmFDL19}, \textit{GmAKT2} expression is reduced in NaCl-treated plants compared with that in NaCl-treated fungus-inoculated plants (Figure 6D). This pattern of expression indicates that during the salt stress, AKT2 expression is downregulated, reducing K\(^+\) transportation through the channel. However, the fungus-inoculated plants showed reduced salt stress and upregulation of AKT2 level compared with those in NaCl-treated plants, which also enhances K\(^+\) transportation. To suppress the stress condition, sufficient concentration of K\(^+\) is required, which could be achieved by the expression of AKT2. \textit{GmSIN1} is involved in salt tolerance and root development, which are achieved by the regulation of ABA and ROS generation (Li et al. 2019). SIN1 alters ABA and ROS levels through the induction of their responsible genes, such as NCED3s and RbohBs, respectively (Li et al. 2019). Our results showed that \textit{GmSIN1} expression was increased under salt stress, whereas the expression level was less in plants under stress condition coupled with fungus inoculation compared with that in pure salt-stressed plants (Figure 6C). This finding indicated that due to the stress mitigation of \textit{Bipolaris} sp., the expression of \textit{GmSIN1} was reduced, which is evident by the induction of \textit{SIN} due to salt stress.

Figure 6. Gene expression in response to the isolate CSL-1 under normal and NaCl stress conditions. Relative expression of (A) \textit{GmFDL19}, (B) \textit{GmNARK}, (C) \textit{GmSIN1}, and (D) \textit{GmAKT2} in soybean plants with and without inoculation of CSL-1 under NaCl stress. The values were calculated relative to those of actin gene expression and are the mean of three replicates. Error bars represent standard errors. The bars with different letters are significantly different from each other as evaluated by DMRT analysis.
Conclusions
This study demonstrated that CSL-1-inoculated plants effectively tolerate a high-saline environment. In soybean plants, CSL-1 enhanced their growth, reduced NaCl toxicity, and inhibited Na\(^+\) uptake and transport. Based on our study findings, we hypothesize that the differences in salt tolerance and accumulation in CSL-1-inoculated soybean plants could be explained by some processes as follows: CSL-1 (i) elevated the antioxidant activity, which could lower ROS concentrations, (ii) enhanced plant growth through its ability to produce IAA and GAs, (iii) provided a physical barrier against salt uptake into the root, and (iv) avoided ionic homeostasis disturbance under salt stress in the inoculated plant. These findings demonstrated that by modulating antioxidant and endogenous phytohormones, CSL-1 significantly reduced the negative effects of NaCl stress damage and enabled soybean plants to grow well. As a result, endophytic isolates CSL-1 that produce GAs and IAAAs, offer a solution for the production of environmentally friendly biofertilizers that boost the growth of economically significant plants under saline environments.

Author contributions
L and MAK conducted the experiments. SA and MW helped in writing the manuscript. MA conducted hormonal and antioxidant analyses. RJ and KMK conducted qRT-PCR analysis. IJL designed, supervised, and financed the research. All authors have read and agreed to its content, and the manuscript also conforms to the journal’s policies.

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Data availability statement
All data generated or analyzed during this study are included in this published article.

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