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Endophytic Fungal Pre-treatments of Seeds Alleviates Salinity Stress Effects in Soybean Plants

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In the present study, four endophytic fungi (GM-1, GM-2, GM-3, and GM-4) were tested for their ability to improve soybean plant growth under salinity stress conditions. The seed germination and plant growth were higher in seeds pretreated with endophytic fungal cultures than their controls. The positive influence of fungi on plant growth was supported by gibberellins analysis of culture filtrate (CF), which showed wide diversity and various concentrations of GAs. Specifically, GA_4, GA_7, GA_8, GA_9, GA_{12}, and GA_{20} were found in fungal CFs. Under salinity stress conditions, GM-1 significantly enhanced the length and fresh weight of soybean plants relative to other fungal treatments. GM-1 effectively mitigated the adverse effects of salinity by limiting lipid peroxidation and accumulating protein content. GM-2, GM-3, and GM-4 also counteracted the salinity induced oxidative stress in soybean plants through reduction of lipid peroxidation and enhancement of protein content, maintaining the length and fresh weight of shoots. The activities of the antioxidant enzymes catalase, superoxide dismutase and peroxidase were inhibited in salinity exposed plants, while GM-1 significantly enhanced these antioxidant enzyme activities in plants under salt stress. GM-1 treatment also showed lower levels of abscisic acid and elevated levels of salicylic acid in plants under salinity stress. Hence, GM-1 was identified as *Fusarium verticillioides* (teleomorph *Gibberella moniliformis*) isolate RK01 based on its DNA sequence homology. These results suggest that endophytic fungal (*F. verticillioides*) pre-treatment of soybean seeds would be an effective method to promote soybean plant growth under salinity stress conditions.

Keywords: antioxidants, endophytic fungi, phytohormones, soybean

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Introduction

Plant growth regulators and chemical fertilizers have been used to increase crop production (Bilkay *et al*., 2010). Application of chemical fertilizers to crop plants negatively affects human health and environments. Recent studies have focused on identification of alternative methods to enhance plant productivity and protect the soil. Soil borne microbes can enter roots and establish their population in plants as endophytes, and many plant-associated fungi are well known for their capacity to promote plant growth; however, the relationship between these microbes and plants is still uncertain (Ikeda *et al*., 2010). Microorganisms have the ability to produce phytohormones, solubilize insoluble phosphate and convert complex organic substances to simple forms. Endophytic fungi have also been shown to impart plants with tolerance to salt, drought, heat and diseases (Waller *et al*., 2005; Khan *et al*., 2011a).

Gibberellic acids (GAs) are secondary metabolites involved in an array of developmental processes including seed germination, dormancy, stem elongation, flowering, sex expression and senescence. The exogenous application of GAs enhances the growth and yield of plants. However, few studies have been conducted to investigate GAs production by endophytic fungi with plant interactions (Hamayun *et al*., 2010). We previously investigated the interaction of GAs producing endophytic fungi with plants under various environmental stress conditions (Khan *et al*., 2011a, 2011b, 2012). In the present study, we confirmed the positive effects of GAs secreting fungi on soybean plants against oxidative stress induced by salinity. Soil salinity is one of the most important factors associated with reduced crop productivity (Chaves *et al*., 2009). Salinity increases free radicals content and disrupts the normal metabolism of proteins, lipids and DNA in plants (Verma and Mishra, 2005). The activation of antioxidant systems such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) in plants can prevent oxidative damage induced by salinity stress. Duan *et al*. (2008) suggested that salinity tolerance of plants was probably achieved by regulation of antioxidant enzymes. In addition, a balanced level of hormones is essential in plant responses to salt stress. Abscisic acid (ABA) biosynthesis is considered to be one of the most important regulators of plant responses to stress (Zhang *et al*., 2006). A number of studies have reported that salicylic acid (SA) is also involved in plants response to abiotic stresses (Alonso-Ramirez *et al*., 2009; Hamayun *et al*., 2010; Khan *et al*., 2011a).

The growth of soybean is affected by various environmental factors including salinity (Radhakrishnan and Ranjitha-Kumari, 2012). Soybean seeds are rich sources of protein,

oil and isoflavonoids; however, the contents of all of these materials were reduced in plants exposed to soil salinity. Currently, environmentally friendly methods for alleviating the toxic effects of salinity on crop plants are the focus of a great deal of agricultural research. The identification and application of beneficial microorganisms to plants can enhance plant growth under salt stress conditions. However, very few studies have investigated the effects of phytohormones secreted from endophytic fungi to improve the growth of crop plants (Khan *et al*., 2011a). Therefore, this investigation was conducted to assess the ameliorative effects of endophytic fungi on soybean against salt stress based on antioxidants and phytohormones analysis.

Materials and Methods

Isolation and mass culture of endophytes from soybean roots

Roots were collected from soybean plants grown in a greenhouse and washed with tap water. For surface sterilization, the roots were subjected to 1% Tween 80 solution, 1% perchloric acid and then thoroughly washed with sterile distilled water for 5 min. The endophytes were isolated according to the procedure described by Hamayun *et al.* (2010). Secondary root pieces were then sliced and inoculated on Hagem medium plates (0.5% glucose, 0.05% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% NH_4Cl, 0.1% $FeCl_3$, 80 ppm streptomycin, and 1.5% agar; pH 5.6±0.2). The fungal colonies were separated individually under sterilized conditions and again cultured on potato-dextrose agar medium plates for further use and storage. The isolated fungal colonies were denoted GM-1, GM-2, GM-3, and GM-4 and cultured in Czapek broth (1% glucose, 1% peptone, 0.05% KCl, 0.05% $MgSO_4 \cdot 7H_2O$, and 0.001% $FeSO_4 \cdot 7H_2O$; pH 7.3±0.2) for 7 days at 30°C while shaking at 120 rpm.

Fungal treatments of soybean seeds

Seeds of soybean (*Glycine max* (L).) cv. Pungsannamulkong were surface sterilized with Teephol and 60% ethanol and then rinsed with sterile distilled water several times. Twenty seeds were then transferred to 20 ml of two weeks old fungal culture of each isolate of GM-1, GM-2, GM-3, and GM-4 and shaken at 120 rpm for 6 h. The soaked seeds were subsequently placed in autoclaved petri dishes containing three layers of filter paper and kept in the dark at 25°C for 3 days, after which distilled water was applied at regular intervals. The plates were observed daily and the speed of germination, peak values, germination values and germination percentage were measured for both control (sterile distilled water and culture medium) and fungal treatments. The germinated seeds were then transferred to pots containing autoclaved substrate composed of peat moss (13–18%), perlite (7–11%), coco-peat (63–68%), zeolite (6–8%), as well as NH^{4+} (~90 mg/kg), NO_3^- (~205 mg/kg), P_2O_5 (~350 mg/kg) and K_2O (~100 mg/kg) (300 g/pot). The shoot length of the plants was measured after 12 days.

Assay of GAs in fungi culture filtrates

Gibberellins secreted from endophytic fungi into the culture medium were measured to determine the diversity of GAs and the variations in their concentration among the four fungal isolates as per the method of Lee *et al.* (1998). Briefly, the culture filtrate (CF) was separated from the mycelium by vacuum filtration with No. 1 Whatman filter papers under controlled conditions. A total of 20 ng of GA standards (obtained from Prof. Lewis N. Mander, Australian National University, Canberra, Australia) was added to each CF. The crude GAs extract in CFs were fractionated using high performance liquid chromatography (HPLC) and the fractions were identified and quantified by gas chromatography/mass spectrometry (GC/MS) with selected ion monitoring (SIM) (6890N Network GC System, and 5973 Network Mass Selective Detector; Agilent Technologies, USA) as described by Khan *et al.* (2011a). The endogenous GAs content of GA_4, GA_7, GA_8, GA_9, GA_{12}, and GA_{20} were calculated from the peak area ratios of 284/286, 222/224, 594/596, 298/300, 300/302, and 418/420, respectively.

Salt treatment of soybean plants

Seventeen day old soybean plants were treated with 300 ml of 0.1 M NaCl for salinity stress induction. Four sets of fungi treated soybean plants were subjected to salinity stress and their ameliorative capacity was assessed by a comparison of untreated control and salinity stressed plants. The aerial parts of plants were harvested to determine the length and weight of shoots, antioxidants and phytohormones at 25 days. All experiments were performed in triplicate.

Assay of lipid peroxidation

Lipid peroxidation was determined according to the method described by Ohkawa *et al.* (1979). Briefly, approximately 0.1 g of fresh leaf samples was homogenized in 10 mM phosphate buffer (pH 7.0) and centrifuged at 10,000 rpm for 15 min at 4°C. The lipid peroxidation of tissue homogenate was then measured based on the malondialdehyde (MDA) content at 532 nm in a spectrophotometer as described by Radhakrishnan and Lee (2013a). Tetramethoxypropane was used as an external standard.

Antioxidant enzymes analysis

Leaf samples (0.5 g) were homogenized in 50 mM Trish HCl buffer (pH 7.0) containing 3 mM $MgCl_2$, 1mM EDTA and 1.0% PVP and then centrifuged at 10,000 rpm for 15 min at 4°C, after which the obtained supernatant was used for assays of the activity of CAT and SOD. All parameters were expressed as the activity per mg protein. The protein concentration of each sample was estimated using the method described by Bradford (1976). The catalase activity was assayed as described by Aebi (1984). Briefly, the crude enzyme extract was added to 0.2 M H_2O_2 in 10 mM phosphate buffer (pH 7.0), after which the CAT activity was determined as a decrease in absorbance at 240 nm and expressed as units (one unit of CAT was defined as μg of H_2O_2 released/mg protein/min).

The activity of peroxidase was measured according to the method described by Kar and Mishra (1976). The crude enzyme source was prepared from leaf samples homogenized with phosphate buffer at pH 6.8 (0.1 M) and centrifuged

Table 1. Effects of endophytic fungal culture on soybean seed germination. Each value is the Mean±SE of six replicates per treatment. Mean values followed by the same letter are not significantly different (P<0.05) as determined by Duncan's multiple-range test.

Treatments	Germination percentage (%)	Germination value	Speed of germination	Peak value	Shoot length (cm)
Con (DW)	70.5±2.6^{d}	389.27±9.20^{c}	10.66±0.6^{ab}	5.51±0.4^{b}	15.56±1.1^{c}
Con (M)	82.3±3.1^{c}	494.52±10.5^{b}	10.66±0.5^{ab}	6.0±0.2^{ab}	14.87±0.5^{d}
GM-1	94.1±2.7^{a}	599.76±12.6^{a}	11.16±0.7^{ab}	6.37±0.3^{a}	18.37±1.9^{a}
GM-2	76.4±4.2^{cd}	299.88±8.61^{d}	05.45±0.3^{c}	3.92±0.5^{d}	17.93±2.1^{b}
GM-3	76.4±3.6^{cd}	365.48±9.65^{cd}	08.12±0.2^{b}	4.77±0.3^{c}	18.06±0.6^{ab}
GM-4	88.2±4.3^{b}	573.09±9.83^{ab}	12.70±0.8^{a}	6.49±0.3^{a}	18.06±0.4^{ab}

DW, Distilled water; M, Culture medium; GM-1, GM-2, GM-3, GM-4 = fungal isolates

at 2°C for 15 min at 17,000 rpm in a refrigerated centrifuge. The aliquot of supernatant was added to phosphate buffer (pH 6.8), 50 µm pyrogallol, 50 µm H_2O_2, and incubated for 5 min at 25°C. The activity of POD was determined based on the absorbance at 420 nm and expressed as changes in absorbance min/mg/protein. An increase in absorbance of 0.1 was considered 1 unit.

SOD activity was assayed as described by Marklund and Marklund (1974) using a reaction mixture containing Tris-HCl buffer (pH 8.2), 2 mM pyrogallol, and distilled water. In this assay, the degree of inhibition of pyrogallol auto-oxidation in crude enzyme extract was measured. The rate of auto-oxidation of pyrogallol was noted every min for 3 min and considered 100% auto oxidation. The enzyme extract was then added to assay mixture containing 2 ml of Tris-HCl buffer (pH 8.2) and 1.5 ml of distilled water, and the reaction was started by adding 0.5 ml of 2 mM pyrogallol. The absorbance at 470 was then immediately read and the SOD enzyme activity was expressed as units (the amount of enzyme utilized to inhibit 50% of auto-oxidation of pyrogallol/ min was calculated as 1 unit).

Abscisic acid (ABA) and salicylic acid (SA) analysis

The ABA content of soybean leaf samples was quantified according to the method described by Qi *et al.* (1998). Crude ABA extract of plant samples was prepared using isopropanol and glacial acetic acid. A total of 10 ng of ABA was added in each extract as an internal standard. The dried extracts were then methylated by adding diazomethane for GC-MS SIM analysis (6890N network GC system, and 5973 network mass selective detector; Agilent Technologies, USA). For quantification, the Lab-Base (ThermoQuest, UK) data system software was used to monitor responses to ions of m/e 162 and 190 for Me-ABA and 166 and 194 for Me-[$^{2}H_6$]-ABA.

The endogenous salicylic acid (SA) analysis in plant samples was performed as described by Enyedi *et al.* (1992) and Seskar *et al.* (1998). Briefly, plant samples were ground with 90% and 100% methanol and then centrifuged at 10,000 rpm for 30 min. The combined methanol extracts were subsequently vacuum-dried and re-suspended in 2.5 ml of 5% trichloroacetic acid, after which the supernatant was partitioned with ethyl acetate: cyclopentane: isopropanol (100:99:1, v/v/v). The top organic layer containing free SA was dried with nitrogen gas and again suspended in 1 ml of 70% methanol. Next, the samples were injected into a C18 reverse-phase HPLC (High Performance Liquid Chromatography) column (HP hypersil ODS, particle size 5 µmol, pore size 120 Å). SA was identified using a fluorescence detector (Shimadzu RF-10AXL, excitation and emission at 305–365 nm, respectively). The flow rate was 1.0 ml/min.

Molecular identification of GM-1 fungi

The fungi isolate, GM-1, was cultured in Czapek broth (1% glucose, 1% peptone, 0.05% KCl, 0.05% $MgSO_4{\cdot}7H_2O$, and 0.001% $FeSO_4{\cdot}7H_2O$; pH 7.3±0.2) for 7 days at 30°C while shaking at 120 rpm. The fugal mycelium was isolated by centrifugation at 5,000 rpm for 30 min. Fungal genomic DNA was extracted from GM-1 using a fungal genomic DNA preparation kit (Solutions for Genetic Technologies, Korea). Endophytic fungal isolate was identified by sequencing the internal transcribed spacer (ITS) rDNA using the universal primers ITS-1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS-4 (5′-TCC TCC GCT TAT TGA TAT GC-3′). The BLAST search program (http://www.ncbi.nlm.nish.gov/BLAST) was used to compare the sequence homology of the nucleotide sequence of the ITS region of related fungi. The GM-1 fungus was identified as *Fusarium verticillioides* based on its 100% sequence homology and submitted to the NCBI database (accession number KC684889).

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the Duncan's multiple range test (DMRT) using SPSS version 11.5 (SPSS Inc., USA). The

Table 2. Gibberellins secretion from soybean endophytic fungi in culture medium. Each value is the Mean±SE of three replicates per treatment. Mean values followed by the same letter are not significantly different (P<0.05) as determined by Duncan's multiple-range test.

CF of fungi	Gibberellins (ng/ml)					
	GA_4	GA_7	GA_8	GA_9	GA_{12}	GA_{20}
GM-1	0.288±0.02^{a}	0.202±0.01^{a}	0.285±0.03^{b}	0.344±0.02^{b}	ND	ND
GM-2	0.036±0.00^{b}	0.015±0.00^{c}	0.332±0.01^{a}	0.149±0.02^{c}	0.550±0.01^{b}	6.203±0.13^{a}
GM-3	ND	ND	0.259±0.01^{c}	0.659±0.03^{a}	0.805±0.04^{a}	ND
GM-4	0.027±0.00^{c}	0.080±0.00^{b}	0.336±0.02^{a}	0.003±0.00^{d}	ND	3.241±0.012^{b}

ND, Not detectable level

values were expressed as the Mean±SE and P values < 0.05 were considered statistically significant.

Results

Role of endophytic fungal culture in soybean seedling growth

Fungal pretreatment significantly enhanced the biological response of soybean seeds by improving seed germination and seedling growth (Table 1). The results showed that application of GM-1 to soybean seeds significantly increased the seed germination and germination value, while the speed of germination and peak value were remarkably enhanced by GM-4 treatment when compared to controls. However, endophytic fungi (GM-1) led to significant increases in seedling shoot length over the control treatments.

The presence of GAs in CF was analyzed using a GC/MS selected ion monitor (SIM) and HPLC. The diversity of GAs in CF varied among endophytic fungi, GM-1 (GA_4, GA_7, GA_8, and GA_9), GM-2 (GA_4, GA_7, GA_8, GA_9, GA_{12}, and GA_{20}), GM-3 (GA_8, GA_9, and GA_{12}), and GM-4 (GA_4, GA_7, GA_8, GA_9, and GA_{20}) (Table 2). Fungal isolate GM-1 produced a higher quantity of GA_4 and GA_7 (0.28 ng/ml and 0.20 ng/ml) and a considerable amount of GA_8 and GA_9 (0.28 ng/ml and 0.34 ng/ml) when compared to other fungi. However, GM-2 secreted more GA_8 and GA_{12}, while GM-3 secreted higher levels of GA_9 and GA_{12} in fungal culture filtrate medium. In addition, we showed a low concentration of GA_4, GA_7, and GA_9 and a higher concentration of GA_8 and GA_{20} in GM-4 culture filtrates.

Effect of endophytes on soybean growth under salinity stress

Soybean plants exposed to endophytic fungi exhibited significant enhancement of plant growth under salt stress conditions. The shoot length and fresh weight of soybean plants decreased under salinity stress (Fig. 1). Fungal isolates, GM-1, GM-3, and GM-4 treatments exhibited increased shoot length under stress condition. The greatest enhancement of shoot length and fresh weight was observed in response to GM-1, followed by GM-4. Protein content was significantly influenced by both salt stress and endophytic fungal isolates (Fig. 1). Salt stress reduced the protein content in soybean plants and elevated levels of protein content were found in plants exposed to GM-1 followed by GM-3, GM-4, and GM-2 relative to the controls.

Lipid peroxidation was higher in plants subjected to salt stress than controls. The endophytic fungi associated seeds (GM-1, GM-3, GM-2, and GM-4) exhibited lower levels of MDA in plants exposed to salinity stress. A significant reduction of lipid peroxidation was observed in response to GM-1 treatment. The growth promoting ability of endophytic fungal isolates on plants under salt stress was verified by an assay of CAT, SOD, and POD (Fig. 2). Activity of CAT was inhibited in plants by the effect of salt induced oxidative stress. GM-2 and GM-4 bio-primed soybean seeds showed decreased catalase activity, while GM-1 showed elevated catalase activity in plants treated with NaCl. However, salt stress decreased the activity of SOD in soybean plants, whereas endophyte GM-2, GM-3, and GM-4 also diminished the SOD activity in salt stressed plants. However, a remarkable increase in SOD activity was found in GM-1 fungus inoculated plants exposed to salt stress. In addition, the maximum level of POD activity was recorded in control plants. Salt stressed plants showed low levels of POD activity, while the plant growth promoting fungi GM-1 induced an increase in POD activity in salt stressed plants. A severe reduction of POD activity was observed in salinity affected plants inoculated with GM-3, GM-2, and GM-4 isolates.

The endogenous ABA content was enhanced in plants treated with NaCl when compared to their controls (Fig. 3). The maximum level of ABA accumulation was found in plants response to GM-4 fungal strains and NaCl treatment. Endophytic fungi, GM-2 and GM-3 inoculated soybean plants also exhibited elevated levels of ABA under salt stress. A significant reduction of endogenous ABA content was re-

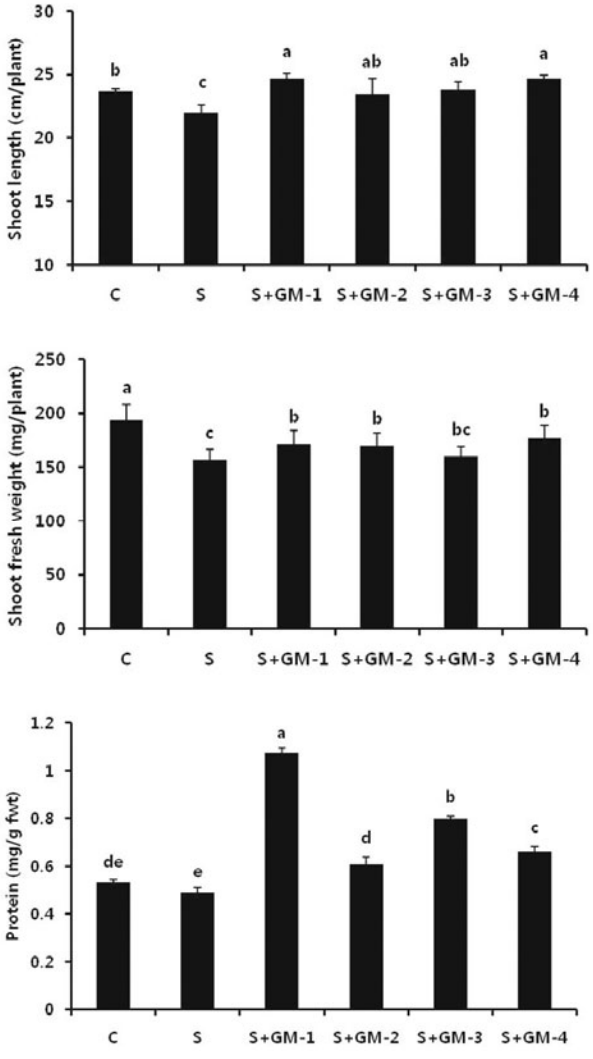

Fig. 1. Effects of endophytic fungal culture (GM-1, GM-2, GM-3, and GM-4) on shoot length, fresh weight and protein content in salt stressed (S) and non-stressed soybean plants (C). Each value is the Mean±SE of six replicates per treatment. Mean values followed by the same letter are not significantly different (P<0.05) as determined by Duncan's multiple-range test.

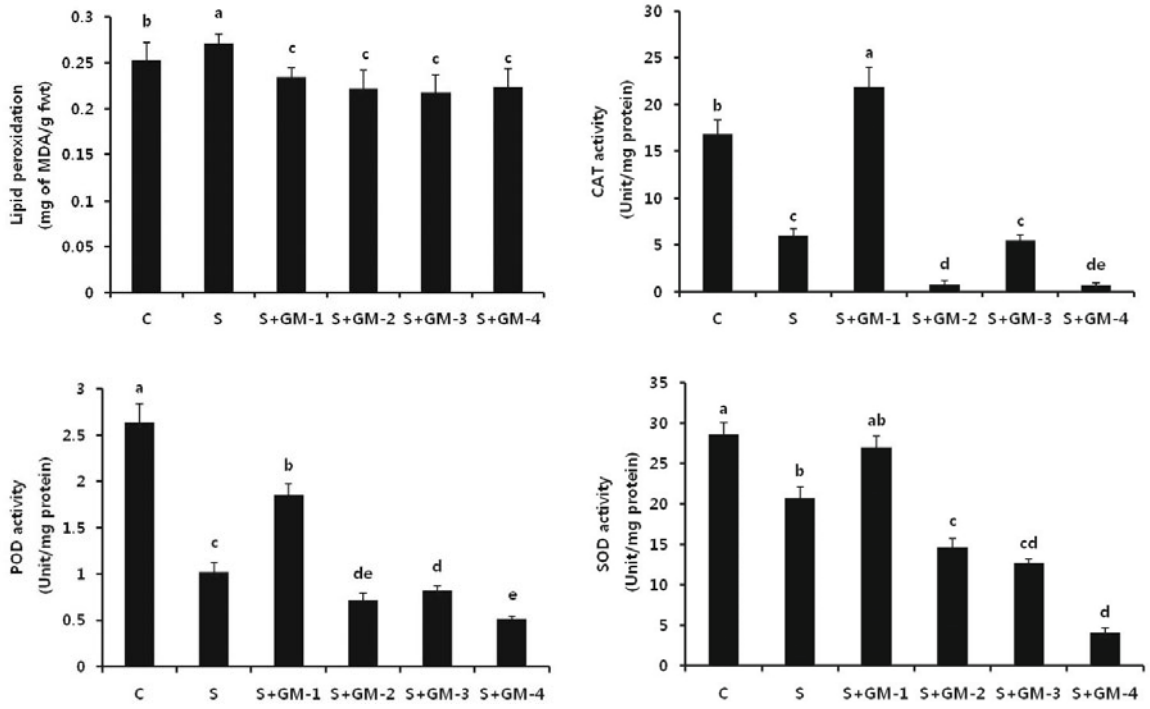

Fig. 2. Effects of endophytic fungal culture (GM-1, GM-2, GM-3, and GM-4) on lipid peroxidation, CAT, POD, and SOD activities in soybean plants exposed with (S) and without (C) salt stress. Each value is the Mean±SE of three replicates per treatment. Mean values followed by the same letter are not significantly different (P<0.05) as determined by Duncan's multiple-range test.

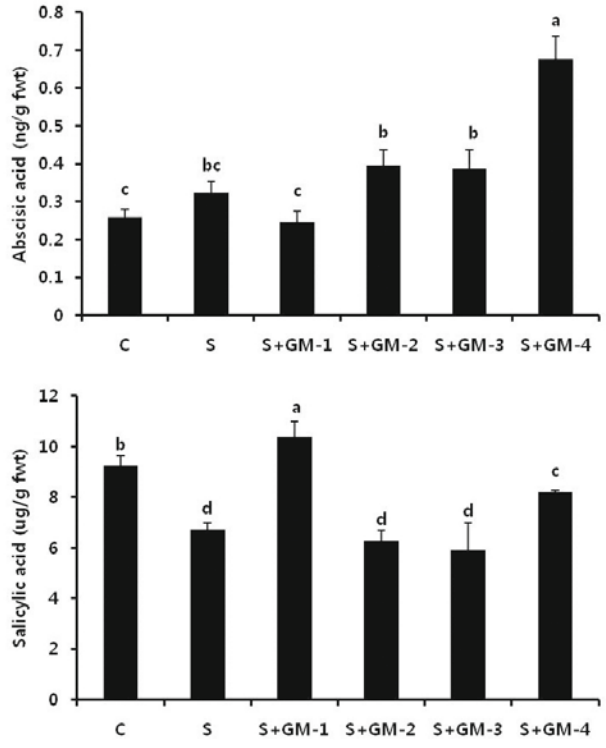

Fig. 3. Effects of endophytic fungal culture (GM-1, GM-2, GM-3, and GM-4) on ABA and SA content in soybean plants exposed with (S) and without (C) salt stress. Each value is the Mean±SE of three replicates per treatment. Mean values followed by the same letter are not significantly different (P<0.05) as determined by Duncan's multiple-range test.

corded in GM-1 bio-primed soybean plants cultivated under saline conditions. Moreover, the endogenous SA was significantly higher in samples treated with salt and GM-3, while other fungal inoculations reduced the SA content in plants exposed to salt and GM-4 followed by GM-1 and GM-2 isolates.

Discussion

The rhizosphere of plants contains a number of microbes that produce secondary metabolites for their survival against biotic and abiotic stresses. Secondary metabolites secreted by fungi, particularly GAs, induce a significant increase in the growth of various crop plants. Endophytic fungi that secrete GAs were identified and their plant growth promoting ability was investigated in a previous study by our group (Hamayun *et al.*, 2010; Khan *et al.*, 2011a; Waqas *et al.*, 2012). The results of a present study demonstrate that the *F. verticillioides* isolate RK01 (GM-1) fungi isolated from soybean root had the ability to produce higher concentrations of GA_4, GA_7, GA_8, and GA_9 than other fungal isolates (GM-2, GM-3, and GM-4). Previously, bioactive and non-bioactive GAs were identified and quantified in culture filtrate of *Aspergillus fumigatus* (Hamayun *et al.*, 2009), *Phoma* sp. (Hamayun *et al.*, 2010), and *Penicillium funiculosum* (Khan *et al.*, 2011a) endophytic fungi. The release of GAs from fungi to rhizosphere or inside the plant root is a source of exogenous GAs that enhances plant growth. In the current study, we observed significant enhancement of soybean seed

germination and seedling growth in response to treatment with GAs producing endophytic fungi. These findings are in agreement with those of Khan *et al.* (2011a), who reported that GA_7, GA_8, GA_9, and GA_{20} produced by *P. funiculosum* could increase plant growth under salt stressed and unstressed conditions. External application of GAs has also been reported to re-programme GAs and other metabolites in plants to improve plant growth (Hamayun *et al.*, 2010; Khan *et al.*, 2011a). Our result suggests that the secretion of GAs from the fungi *F. verticillioides* isolate RK01 (GM-1) might be a reason for improving soybean plant growth.

Salinity adversely affects the growth of crop plants; however, mycorrhizal fungal associations have shown improved plant tolerance to saline environments (Porcel *et al.*, 2012). The secretion of plant growth promoting compounds (GAs and IAA) from endophytic fungi is a reason for enhanced plant growth under abiotic stress conditions (Khan *et al.*, 2011a, 2012). Soybean plants inoculated with GM-1 (*F. verticillioides* isolate RK01) showed a significant increase in length and fresh weight of shoots under saline stress. *F. verticillioides* is an endophytic fungi that plays a dual role as either beneficial or harmful in host plants, while pretreatment of seeds with *F. verticillioides* is known to enhance maize plant growth and yield, even under biotic and abiotic environments (Yates *et al.*, 2005). Previous studies also demonstrated that application of mycorrhizal fungi can counteract the detrimental effects of salt stress on soybean (Khan *et al.*, 2011a), bajra (Borde *et al.*, 2011), tomato (Hajiboland *et al.*, 2010), citrus (Wu *et al.*, 2010), and lettuce (Kohler *et al.*, 2009) plants. Recently, we reported the active colonization of endophytic fungi (*Penicillium minioluteum*) with host plant (soybean) roots at before and after salt stress treatments (Khan *et al.*, 2011b).

Soil salinity induces ROS accumulation in plants, which damages DNA, proteins and lipids in plant cells (Fridovich, 1986). In addition, the patterns of gene expression and protein synthesis in plants are affected by oxidative stress (Akhter *et al.*, 2004). Inoculation of mycorrhizal fungi increased the protein content in salt affected plants (Tunc-Ozdemir *et al.*, 2009). In our experiment, we also found a decline in protein content and an increase in lipid peroxidation in plants subjected to salt stress, while a drastic enhancement of protein content along with a reduced level of lipid peroxidation was observed in salt affected plants pretreated with GM-1 (*F. verticillioides* isolate RK01). These changes were followed by those observed in response to treatment with GM-3, GM-4, and GM-2. Reduction of lipid peroxidation might have helped to increase the salt tolerance of plants (Juan *et al.*, 2005). The higher level of lipid peroxidation in plants decreases membrane fluidity, membrane proteins and enzymes (Gill and Tuteja, 2010). In addition, the lower level of lipid peroxidation induced by mycorrhizal fungi indicates lower oxidative damage in plants in salt stress environments (Hajiboland *et al.*, 2010; Latef and Chaoxing, 2011).

The role of antioxidant enzymes against oxidative stress in plants has been widely reported by many researchers (Garratt *et al.*, 2002; Hernandez *et al.*, 2003; Radhakrishnan *et al.*, 2012; Radhakrishnan and Lee, 2013a, 2013b). CAT, POD, and SOD are important antioxidants that eliminate reactive oxygen species. SOD catalyzes O_2^- to H_2O_2, whereas CAT converts H_2O_2 to water and oxygen. Khedr *et al.* (2003) reported that salt stress inhibited the activities of catalase and peroxidase in *Pancratium maritimum* (L.) plants. In the present study, a decrease in CAT, POD, and SOD activities was observed in soybean plants under saline conditions. Mycorrhizal fungi have the ability to enhance antioxidants in plants under abiotic stress. For example, *Glomus mosseae* is an arbuscular mycorrhizal fungus that was found to protect citrus seedlings from the effects of salinity stress via stimulation of antioxidant defense systems (Wu *et al.*, 2010). We found significantly higher CAT, POD, and SOD activities in GM-1 (*F. verticillioides* isolate RK01) treated plants under salinity conditions when compared to non-salt stressed conditions. Our results are partially correlated with those of Alguacil *et al.* (2003), who found that inoculation of mycorrhizae increased CAT activity in *Oleo europaea* cultivated under semi-arid conditions. In addition, higher levels of POD also protect plants against the oxidative stresses (Sreenivasulu *et al.*, 1999). Pacovsky *et al.* (1991) observed an increase in POD activity in *Phaseolus vulgaris* treated with *Glomus etunicatum*. The mycorrhizal symbiosis might be increase SOD and POD in salinity injured plants (He *et al.*, 2007).

The phytohormone ABA is a stress related signaling molecule that responds to biotic and abiotic stresses (Ben-Ari, 2012). Several studies have demonstrated an increase in ABA content in soybean plants under salt and osmotic stress conditions (Khan *et al.*, 2011a; Radhakrishnan and Lee, 2013a, 2013b). Exogenous application of endophytic mycorrhizal fungi mitigated the salt stress effects in plants via a reduction of ABA content (Hamayun *et al.*, 2010; Khan *et al.*, 2011a). The current study also showed significantly lower ABA content in salt affected plants pretreated with the endophytic fungus, *F. verticillioides* isolate RK01 (GM-1), relative to salt stressed plants, suggesting that regulation of ABA by GM-1 might enhance soybean plant growth in saline environments. In addition, the defense role of SA in plant responses to abiotic stresses is well known (Borsani *et al.*, 2001; Iqbal and Ashraf, 2010). This role involves acting as an ROS scavenger in stressed plants (Baek *et al.*, 2010). The interaction of fungi with plants showed various degrees of SA expression in crop plants (Hamayun *et al.*, 2010; Khan *et al.*, 2012). In the current study, we found a marked increase of SA content in soybean plants treated with GM-1 under salt stress conditions. The ameliorative effects of SA against the adverse effects of salinity might be due to the enhanced activation of defensive enzymes and photosynthetic pigments (Idrees *et al.*, 2012).

Conclusion

The ability of gibberellins production and plant growth promotion of endophytic fungi with multifunctional properties has received a great deal of attention in the biofertilizer field. *F. verticillioides* isolate RK01 (GM-1) is a gibberellins secreting endophytic fungi that can ameliorate salt stress effects through regulation of antioxidants and endogenous phytohormones. These findings indicate that seed pre-treatment with endophytic fungi would be useful for sustainable agri-

culture to improve crop plant growth under salt stress conditions.

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