

# A comparative study of phosphate solubilization and the host plant growth promotion ability of *Fusarium verticillioides* RK01 and *Humicola* sp. KNU01 under salt stress

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**Abstract** The aim of the present study was to compare the benefit to soybean in terms of phosphate solubilization and host plant growth under salt stress of the endophytic fungi *Fusarium verticillioides* RK01 and *Humicola* sp. KNU01. Endophyte *F. verticillioides* RK01 increased phosphate solubilization significantly more than *Humicola* sp. KNU01, as confirmed by increasing activities of acid phosphatase ( $1.0 \text{ U}^{-1} \text{ g fwt}$ ), alkaline phosphatase ( $2.1 \text{ U}^{-1} \text{ g fwt}$ ) and fungal biomass (4.6 g), with a concurrent decrease in tricalcium phosphate concentration in the culture medium. In a plant growth evaluation study, 0.1 M NaCl was added to pots containing soybean plants pre-treated with fungi. The salt-affected plants exhibited stunted growth due to a lower concentration of protein and higher level of lipid peroxidation than their controls. *F. verticillioides* RK01 and *Humicola* sp. KNU01 isolates significantly increased shoot length (7.3 % and 2.5 %) and protein content (59.1 % and 65.1 %), while inhibiting the lipid peroxidation level in salt stressed plants. Moreover, carotenoids, the activities of superoxide dismutase (SOD) and catalase were lower in plants affected by salinity than in non-stressed plants, whereas *Humicola* sp. KNU01 fungal association significantly mitigated oxidative stress by enhancing carotenoids (24.1 %) and SOD activity (61.2 %). *F. verticillioides* RK01 also triggered SOD activity to prevent oxidative damage. In addition, salt stress caused an increase of abscisic acid (ABA) in soybean plants, and *F. verticillioides* RK01 colonization effectively reduced ABA content in stressed plants. Salicylic acid (SA) is another plant hormone that was present in lower amounts in stressed plants, but the endophytic (*F. verticillioides* RK01 and *Humicola* sp. KNU01)

fungal association increased SA content in salt-affected plants. The results of the present study indicate that the efficient phosphate solubilizing endophytic fungi *F. verticillioides* RK01 was more favorable to protect soybean plants against oxidative damage than *Humicola* sp. KNU01.

**Keywords** Antioxidants · Endophytic fungi · Salt stress · Soybean · Stress hormones

## Introduction

Microorganisms have the ability to solubilize insoluble phosphate (Pradhan and Sukla 2005), and also to produce phytohormones. The inoculation of phosphate solubilizing endophytic fungi into soil is a new approach to increase plant growth even under unfavorable salt stress conditions (Khan et al. 2011a). Phosphorous is present in soil at 20–80 % as inorganic forms with calcium, iron and aluminium; or in organic forms (Richardson 2001) that derive from decaying living organisms (Pandey et al. 2008). Moreover, very low quantities of phosphate (0.1 %) are present in a form accessible to plant uptake (Hariprasad and Niranjana 2009). Phosphate is an important macro nutrient, plays a role in plant bioenergetics, and is needed for converting light energy to ATP during photosynthesis, phosphorylation, cell signaling, and synthesis of biomolecules. Phosphate also leads to stimulation of plant growth, flower and seed formation. The existence of phosphate-solubilizing microorganisms in soil has led to the belief that secretion of microbial metabolites such as organic acids plays a key role in converting insoluble phosphate into soluble forms, and to the suggestion that the application of encapsulated soil microorganisms might enhance phosphate solubilization (Fenice et al. 2000; Vassilev et al. 2001). Moreover, Vassilev et al. (2006) documented that

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*Aspergillus* and *Penicillium* effectively solubilize rock phosphate, and that the fungal association enhances the uptake capacity of phosphorous by plants (Alvarez et al. 2009) and, thus, phosphate-solubilizing microorganisms are helpful for sustainable agriculture (Khan et al. 2007).

The mutual interaction of endophytic fungi and plants may differ in different hosts and environmental conditions (Fateh and Fagan 2002). Root-associated endophytes can improve the growth and yield of crop plants by regulating physiological process such as water- and nutrient- uptake (Auge 2001; Wu et al. 2010). Such endophytes are ubiquitous among the large group of soil microorganisms that are known to exist in saline environments (Giri et al. 2003). Soil salinity is one of the important factors limiting plant growth and yield. Endophytic fungi secrete a wide array of bioactive metabolites including gibberellins and indole acetic acid (Khan et al. 2011a), and the role of these plant growth regulators is well known for growth and development of plants under stress conditions. Poss et al. (1985) documented that fungal association with roots improves the salt tolerance of plants.

Soybean is an economically valuable crop, and the yield of this plant is reduced by high salinity. The exogenous application of various agro chemicals and seed pre-treatments may alleviate the oxidative damage induced by salt stress (Radhakrishnan et al. 2012). Recently, several studies have focused on the use of endophytic fungi to prevent salt stress effect in plants, and results suggest that biological organisms can be used as an alternative to chemical fertilizers (Ghorbanli et al. 2004; Khan et al. 2011a; Sohrabi et al. 2012a; Wu et al. 2013). A number of beneficial microorganisms have been isolated from soil and plant roots, and their plant growth promoting ability has also been well demonstrated in crop plants, but still very few studies have documented or elucidated the role in plants of endophytic fungi in stress regulatory metabolism against abiotic stress (Khan et al. 2012). In the present study, we investigated the mitigating effect of endophytic fungi (*F. verticillioides* RK01 and *Humicola* sp. KNU01) on oxidative stress-related physiological changes such as plant hormones and antioxidant activities in stressed plants. The aim of the current study was to evaluate the phosphate solubilization ability of two endophytic fungi, and their mitigating effects on soybean plants under salt stress conditions.

## Materials and methods

### Isolation and identification of endophytes from soybean roots

The endophytic fungi were isolated from roots of soybean plants grown in a greenhouse according to the method described by Khan et al. (2011a). The roots were separated from plants and washed thoroughly with tap water followed by

Tween 80 solution, 1 % perchloric acid and sterilized distilled water. The root samples (0.5 cm) were placed on Hagem medium plates (0.5 % glucose, 0.005 %  $\text{KH}_2\text{PO}_4$ , 0.05 %  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.05 %  $\text{NH}_4\text{Cl}$ , 0.1 %  $\text{FeCl}_3$ , 80 ppm streptomycin and 1.5 % agar; pH  $5.6 \pm 0.2$ ), and incubated at 25 °C. The fungal colonies were separated and re-inoculated on potato dextrose agar plates. The two fungal colonies were cultured individually on Czapek broth (1 % glucose, 1 % peptone, 0.05 % KCl, 0.05 %  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and 0.001 %  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ; pH  $7.3 \pm 0.2$ ) for 7 days at 30 °C with 120 rpm. For identification of fungi, genomic DNA was isolated from each isolate as described by Khan et al. (2011a), and identified by sequencing the internal transcribed spacer (ITS) rDNA, using universal primers ITS-1 (5'-TCCGTAAGTGAACCTG CGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3'). The BLAST search program (<http://www.ncbi.nlm.nih.gov/BLAST>) was used to compare the nucleotide sequence homology of the 18S ITS region of fungi. The two fungal isolates were identified as *Fusarium verticillioides* isolate RK01 (accession number KC684889) and *Humicola* sp. KNU01 (accession number KC797230).

### Phosphate solubilization ability of fungi

The two fungal strains (*F. verticillioides* RK01 and *Humicola* sp. KNU01) were tested for their capacity to solubilize tricalcium phosphate. For estimation of phosphate solubilization activity, 100 mL medium (PD broth, adjusted to pH 7.0) containing 0.5 % of tricalcium phosphate (w/v) was inoculated with 100  $\mu\text{L}$  fungal sample (ca.  $30 \times 10^3$  CFU/mL) and incubated at 28 °C for 30 days (Kang et al. 2002). The tricalcium phosphate and pH in culture filtrates, as well as fungal biomass and acid phosphatase and alkaline phosphatase activities were recorded at 10- and 30-day intervals. Briefly, fungal culture was centrifuged at 7,000 rpm for 20 min after measuring the pH, and tricalcium phosphate was estimated in the culture supernatant at 600 nm in a spectrophotometer according to King (1936) with some modifications. The pellet of fungal mycelial samples was transferred to a pre-weighed Whatman No.1 filter paper, and biomass was measured. For the analysis of phosphates activity, fungi were ground separately with 0.1 M acetate buffer (pH 5.0) for acid phosphatase or with 0.1 M Tris-HCl buffer (pH 8.2) for alkaline phosphatase, as a source of crude enzyme. Activity of acid and alkaline phosphatase was measured according to the modified method of Ikawa et al. (1964).

### Fungal interaction with soybean plants under salt stress

Two-week-old fungal cultures of *F. verticillioides* RK01 and *Humicola* sp. KNU01 were applied to 15-day-old soybean seedlings grown on substrate containing peat moss (13–18 %, w/v), perlite (7–11 %, w/v), coco-peat (63–68 %, w/v) and

zeolite (6–8 %, w/v) and ~90 mg/kg  $\text{NH}_4^+$ , ~205 mg/kg  $\text{NO}_3^-$ , ~350 mg/kg  $\text{P}_2\text{O}_5$ , and ~100 mg/kg  $\text{K}_2\text{O}$ . Pots treated with sterile water served as a control. The plants were grown in a greenhouse under a day/night cycle of 16/8 h and at 28/20 °C and 60 % relative humidity. For salt stress induction, 300 mL 0.1 M NaCl was applied to soybean plants at 33 days. Shoot length and biochemical content were analyzed in 38-day-old soybean plants. Six replicates were used in each treatment.

#### Oxidative damage to lipids

Lipid peroxidation of leaf samples was estimated in terms of amount of malondialdehyde (MDA) content according to the method of Ohkawa et al. (1979). The reaction mixture consisted of 0.2 mL 10 mM phosphate buffer (pH 7.0), leaf extract, 0.2 mL 8.1 % sodium dodecyl sulphate, 1.5 mL 20 % acetic acid (pH 3.5) and 1.5 mL 0.81 % thiobarbituric acid aqueous solution. The mixture was heated in boiling water for 60 min, and 5 mL butanol:pyridine (15:1 v/v) solution was added at room temperature. The absorbance of the upper organic layer was read at 532 nm. Tetramethoxypropane was used as an external standard. The concentration of MDA was calculated from standard graph values and expressed as milligrams of MDA formed per gram of fresh weight.

#### Protein content

Protein content in leaf samples was determined according to the method of Bradford (1976). Leaf samples were homogenized in 50 mM Tris-HCl buffer (pH 7.0), 3 mM  $\text{MgCl}_2$ , 1 mM EDTA and 1.0 % PVP and centrifuged at 10,000 rpm for 15 min. The protein content of supernatant (or crude enzyme extract) was measured at 595 nm using a spectrophotometer.

#### Catalase activity

Catalase (CAT) activity was assayed by the method of Aebi (1984); crude enzyme extract was added to 0.5 mL 0.2 M  $\text{H}_2\text{O}_2$  in 10 mM phosphate buffer (pH 7.0). CAT activity was estimated by the decrease in absorbance of  $\text{H}_2\text{O}_2$  at 240 nm (1 unit of CAT activity was defined as that amount of the enzyme that breaks down 1  $\mu\text{mol}$   $\text{H}_2\text{O}_2$  per minute under assay conditions).

#### Superoxide dismutase activity

Superoxide dismutase (SOD) activity was estimated by measuring the degree of inhibition of pyrogallol auto-oxidation by crude enzyme extract, according to Marklund and Marklund (1974). The reaction mixture for auto-oxidation consisted of 2 mL Tris-HCl buffer (pH 8.2), 0.5 mL 2 mM pyrogallol and 2 mL distilled water. Initially, the rate of auto-oxidation of

pyrogallol was noted every minute for 3 min. This was considered as 100 % auto oxidation. The assay mixture for the enzyme contained 2 mL Tris-HCl buffer pH 8.2, 1.5 mL distilled water, 0.5 mL supernatant and 0.5 mL 2 mM pyrogallol. The absorbance was read at 470 nm. The enzyme activity was expressed in units. One unit of SOD activity was the amount of enzyme needed to inhibit 50 % of auto-oxidation of pyrogallol per minute.

#### Abscisic acid content

The endogenous abscisic acid (ABA) content was extracted from leaf tissue and estimated according to the method of Qi et al. (1998). The extracts were dried and methylated by adding diazomethane for GC-MS SIM (6890 N network GC system, and 5973 network mass selective detector; Agilent Technologies, Palo Alto, CA) analysis. For quantification of ABA, Lab-Base data system software (ThermoQuet, Manchester, UK) was used to monitor responses to ions of m/e 162 and 190 for Me-ABA and 166 and 194 for Me-[ $^2\text{H}_6$ ]-ABA.

#### Salicylic acid content

Salicylic acid (SA) was extracted and quantified by method of Enyedi et al. (1992) and Seskar et al. (1998). Freeze dried plant samples were homogenized with 90 % and 100 % methanol by centrifuging at 10,000 rpm. The combined methanol extracts were vacuum-dried. Dry pellets were re-suspended in 2.5 mL 5 % trichloroacetic acid, and 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 70 % methanol. The samples were injected to C18 reverse-phase high performance liquid chromatography (HPLC) column (HP hypersil ODS, particle size 5  $\mu\text{mol}$ , pore size 120 Å). SA was identified by fluorescence detector (Shimadzu RF-10AXL, excitation and emission at 305–365 nm, respectively). The flow rate was 1.0 mL/min.

#### Statistical analysis

The experiments were performed in a completely randomized design. Data were subjected to statistical analysis using SPSS software version 11.5 (SPSS, Cary, NC). The significant differences among the mean values of treatments ( $n=3$ ) were determined using DMRT test at  $P<0.05$  level.

## Results and discussion

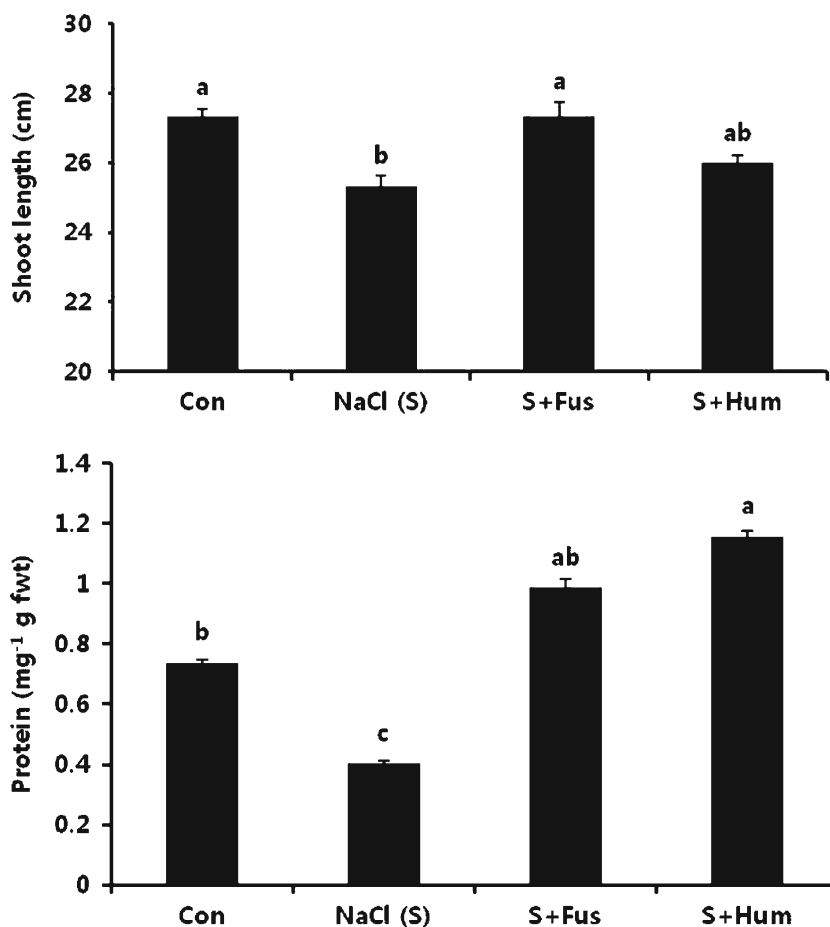
Soil contains a wide range of microbes including fungi; beneficial mycorrhizal fungi, which live in either soil or plant

**Table 1** Phosphate solubilization ability of *Fusarium verticillioides* RK01 and *Humicola* sp. KNU01 endophytic fungi grown in liquid medium. Values are means plus standard error ( $n=3$ ). Means followed by the same letter (a and b) are not significantly different ( $P<0.05$ ) as determined by DMRT test

Fungal isolate	10 days			30 days				
	Biomass (g)	pH	Phosphate (mg/L)	Biomass (g)	pH	Phosphate (mg/L)	Acid phosphatase (U)	Alkaline phosphatase (U)
<i>F. verticillioides</i> RK01	7.199±0.5 a	5.31±0.2 a	0261.9±1.6 b	4.608±0.7 a	5.94±0.1 a	0016.3±0.0 b	1.00±0.06 a	2.12±0.03 a
<i>Humicola</i> sp. KNU01	0.244±0.0 b	4.98±0.2 b	2351.6±8.4 a	4.422±0.2 a	4.88±0.3 b	2263.6±7.8 a	0.10±0.00 b	0.34±0.00 b

roots as endophytes, play a vital role in improving plant growth. In the current study, two endophytic fungi were isolated from soybean roots and identified as *F. verticillioides* RK01 and *Humicola* sp. KNU01 based on their ITS region and phylogenetic analysis. The phosphate solubilization ability of these endophytic fungi was assessed at 10 and 30 days, and corresponding changes in biomass of fungi, pH and phosphate concentration of broth culture were determined (Table 1). *F. verticillioides* RK01 isolate exhibited a higher amount of biomass (7.19 g) than *Humicola* sp. KNU01 isolate. A reduction in pH was observed in 10- and 30-day-old fungal cultures (*F. verticillioides* RK01 and

*Humicola* sp. KNU01) compared to the initial pH of the medium (pH 7.0). This indicates an increase in gluconic acid production and phosphate solubilization in the medium (Reyes et al. 1999). The higher level of phosphate solubilization ability of the *F. verticillioides* RK01 isolate was evidenced from the low concentration of tri-calcium phosphate (0.26 mg and 0.001 mg at 10 and 30 days, respectively) in the medium. Secretion of phosphatase enzymes, secondary metabolites and organic acids from fungi is a common mechanism, which induces the conversion of insoluble forms of phosphorous to plant-available forms (Jha et al. 2009). Acid and alkaline phosphatase play significant roles in fungi in the utilization

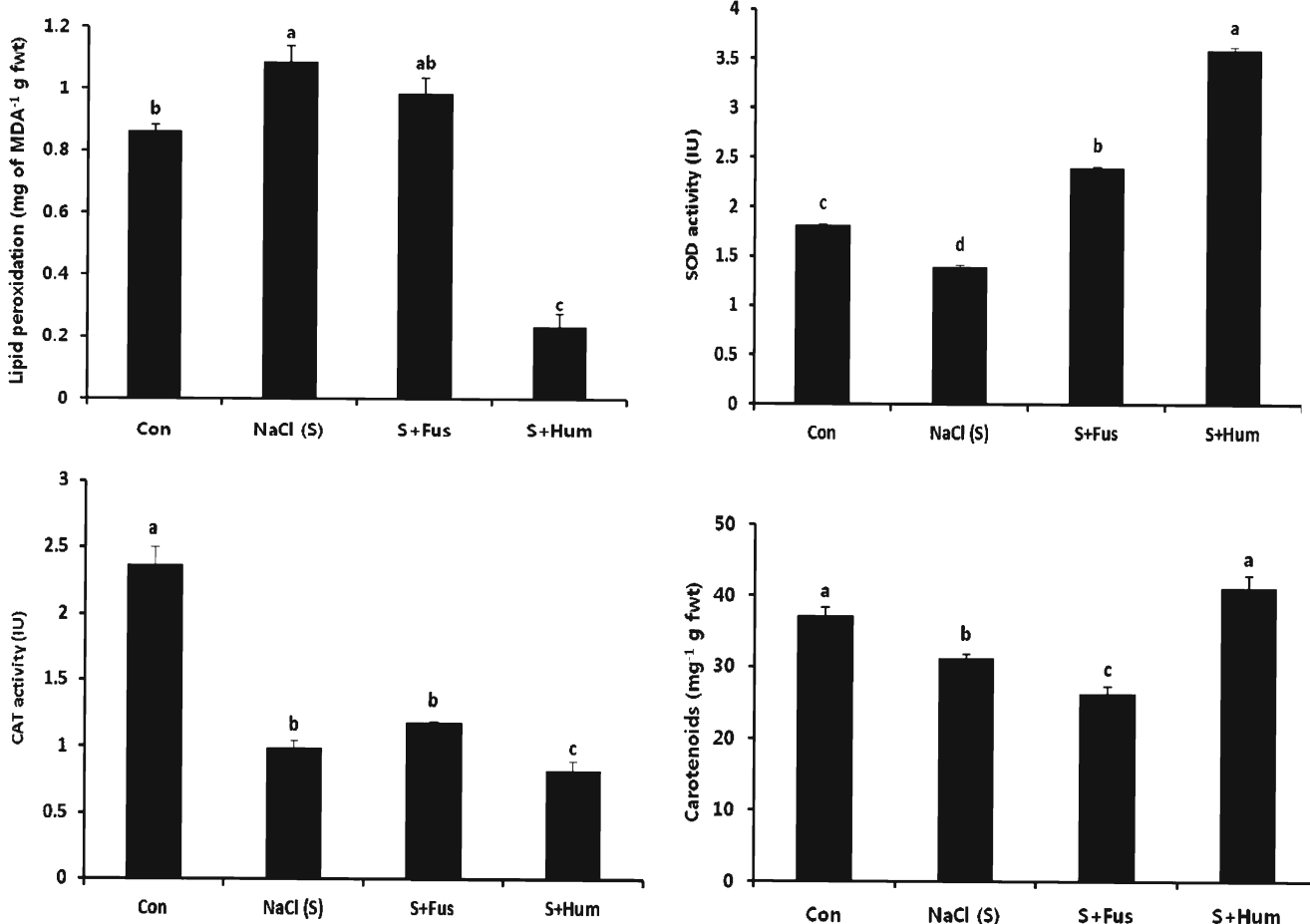
**Fig. 1** Influence of *Fusarium verticillioides* RK01 (Fus) and *Humicola* sp. KNU01 (Hum) on soybean shoot length and protein content in NaCl salt (S)-injured plants. Bars Means plus standard error. Means followed by the same letter are not significantly different ( $P<0.05$ ) as determined by DMRT test

of organic phosphate from the soil. Both acid and alkaline phosphatase activities were higher in endophytic fungus *F. verticillioides* RK01 than in *Humicola* sp. KNU01 (Table 1).

The mutualistic interaction of endophytic fungi favors improvement in the plant growth and development, and can enhance stress tolerance of plants grown under unfavorable abiotic stress environments. Soil salinity limits plant growth, but the host plant is protected by endophytes and their biologically active secondary metabolites (Khan et al. 2011b). Previously, the ameliorative role of mycorrhizal fungi against salt stress in lettuce, citrus, tomato and bajra was documented by Kohler et al. (2009), Wu et al. (2010), Hajiboland et al. (2010) and Borde et al. (2011). In the present study, salt-stress-affected soybean plants showed a significant reduction in their shoot length (7.9 %); this reduction in growth was ameliorated by *F. verticillioides* RK01 and *Humicola* sp. KNU01, which increased shoot length by 7.3 % and 2.5 %, respectively (Fig. 1). A remarkable level of stress alleviation was found in *F. verticillioides* RK01-associated plants. Yates et al. (2005) reported that pretreatment of seeds with *F. verticillioides*

enhanced maize plant growth and yield, under both biotic and abiotic environmental conditions. The important contribution of *F. verticillioides* RK01 fungal association to the improvement of plant growth might be due to an increase in uptake of phosphorus and other elements by host plants (Wu et al. 2010). Previous findings also revealed that *Glomus* sp., *Diversispora* sp. and *Exophiala* sp. fungal interaction could improve host plant growth under abiotic stresses (Khan et al. 2011c; Sohrabi et al. 2012a; Sheng et al. 2009), suggesting that inoculation of plants with mycorrhizae can enhance plant growth by increasing the water- and nutrient- absorption capacity of plants (Auge 2001). In addition, salt stress significantly diminished the protein content (82.9 %) in non-fungal associated plants, while endophytic fungal (*F. verticillioides* RK01 and *Humicola* sp. KNU01)-associated plants exhibited a higher protein content under stress conditions (Fig. 1). Sohrabi et al. (2012b) also observed a higher level of protein in drought-stressed chickpea plants inoculated with *Glomus* sp. than in stressed plants.

Accumulation of ROS in plant cells under environmental stress conditions is a major factor causing damage to lipids,



**Fig. 2** Effect of *F. verticillioides* RK01 (Fus) and *Humicola* sp. KNU01 (Hum) on lipid peroxidation, carotenoids, superoxide dismutase (SOD) and catalase (CAT) activities in plants exposed to NaCl salt (S) stress.

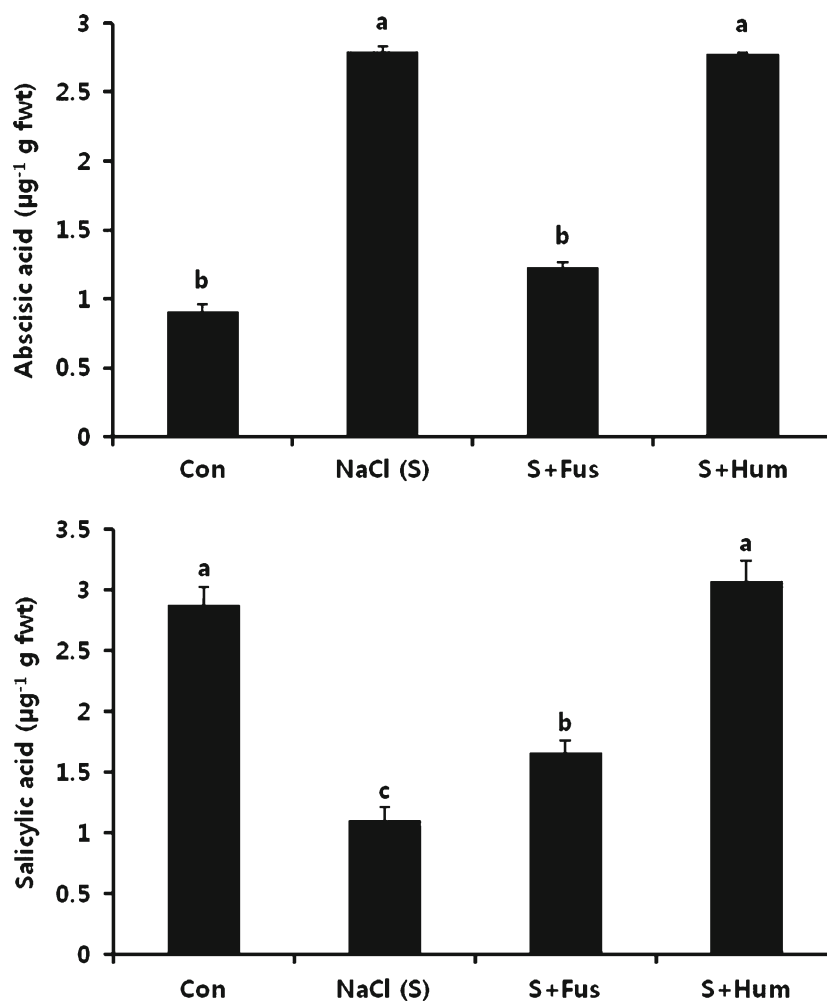
Bars Means plus standard error ( $n=3$ ). Means followed by the same letter are not significantly different ( $P<0.05$ ) as determined by DMRT test

proteins and nucleic acids (Imlay and Linn 1988; Jiang and Zhang 2001). Salt stress causes detrimental effects on plants by increasing lipid peroxidation (MDA content) and reducing antioxidant activities (Fig. 2). High concentrations of MDA in plant cells are considered a stress indicator, damaging membrane lipids and leading to inactivation of receptors, enzymes and ion channels (Gill and Tuteja 2010; Radhakrishnan et al. 2012). Exogenous application of endophytic fungi, *F. verticillioides* RK01 and *Humicola* sp. KNU01 reduced the MDA level in salt-stressed plants (Fig. 2), and a significant three-fold reduction of MDA content (0.2 mg/g fwt) was observed in *Humicola* sp. KNU01 fungi-associated salt-affected plants. These results suggest that endophytic fungi protects host plants by avoiding soil-salinity-induced oxidative stress damage.

Next, the mitigating effect of *F. verticillioides* RK01 and *Humicola* sp. KNU01 against salinity stress was further assessed by measuring antioxidant activities. It is widely known that ROS metabolism is correlated with antioxidant molecules and enzymes (Radhakrishnan and Lee 2013); however, there is little information on the protective effect of

mycorrhizal colonization on plant enzymatic antioxidant defense systems (Ghorbanli et al. 2004). Plants are able to prevent the cytotoxic effects of ROS by using antioxidants such as SOD, CAT and carotenoids (Sharma and Dubey 2005). In the current study, we found a reduction (19.2 %) in the level of antioxidants such as carotenoids, and in the activities of CAT (58.1 %) and SOD (23.7 %) in salt-stressed plants compared to controls (Fig. 2). Under stress conditions, superoxide anion ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ) production are higher in plants, and self defense mechanisms such as SOD and CAT are activated to prevent oxidative damage (Radhakrishnan and Lee 2013). SOD converts  $O_2^-$  content to  $H_2O_2$  and water, whereas CAT dismutates  $H_2O_2$  to water and oxygen. A significant higher SOD activity was found in *F. verticillioides* RK01- and *Humicola* sp. KNU01-inoculated plants than in fungal free control plants even under stress conditions (Fig. 2). These results reflect the fact that endophytic fungal association avoids stress effects by stimulating SOD activity to scavenge ROS. However, *F. verticillioides* RK01 and *Humicola* sp. KNU01 fungi inhibited CAT activity in salt-stressed plants. The mechanisms

**Fig. 3** *Fusarium verticillioides* RK01 (Fus)- and *Humicola* sp. KNU01 (Hum)-induced changes in abscisic acid (ABA) and salicylic acid (SA) content in soybean plants under NaCl salt (S) stress conditions. Bars Means plus standard error ( $n=3$ ). Means followed by the same letter are not significantly different ( $P<0.05$ ) as determined by DMRT test



of antioxidant systems in plant cells are complex, and fluctuate under an array of stresses. On the other hand, carotenoids are pigment molecules involved in photosynthesis that also act as antioxidants in plants to prevent the photo-oxidative damage generated by biotic and abiotic factors (Siefermann-Harms 1987). We found that *Humicola* sp. KNU01 fungal association increased the level of carotenoids in salt-stressed plants (Fig. 2), while *F. verticillioides* RK01 fungal interactions with soybean under salt-stressed conditions showed a lower concentration of carotenoids than stressed plants. Therefore, the elevated content of carotenoids and SOD activity in *Humicola* sp. KNU01-associated plants might indicate that this fungus had an efficient capacity to avoid oxidative stress.

The response of plants to salt stress is initiated at the receptors in membranes, and results in the production of ROS, ABA and other secondary signaling molecules (Porcel et al. 2012). ABA protects plants from abiotic stresses through the regulation of many stress-responsive genes and stomatal closure to minimize water loss (Zhang et al. 2006). It has been widely documented that accumulation of ABA content in plants increases under salt stress conditions, while fungal association with plants can regulate ABA synthesis (Jahromi et al. 2008; Khan et al. 2012; Porcel et al. 2012). The findings of the current study showed higher accumulation of ABA in plants exposed to salt stress than in controls (Fig. 3). The *F. verticillioides* RK01 isolate ameliorated the stress effect in soybean by reducing ABA content, while *Humicola* sp. KNU01 fungal association had no effect on ABA content in plants under salt stress. Khan et al. (2011b) reported a lower concentration of ABA in salt-injured soybean plants colonized by *Penicillium minioluteum* than in non-fungal treated plants. There is also evidence that levels of ABA altered by fungal association induces salt stress tolerance in soybean. However, the effect of mycorrhizae on endogenous ABA content in plants seems contradictory (Dodd and Perez-Alfocea 2012), and Evelin et al. (2009) suggested that ABA accumulation might respond differentially to the influence of different mycorrhizal fungal species. In our study, we also found that *Humicola* sp. KNU01 fungal association had no effect on ABA content.

SA is a signaling molecule that plays an important role in plant response to abiotic stresses (Alonso-Ramirez et al. 2009) and that is necessary to generate antioxidants (Sharma et al. 1996). The mutual interaction of fungi and plants increases SA production in induced systemic resistance against abiotic stresses, and high endogenous SA can mitigate the negative effects of ROS accumulation (Khan et al. 2012). Although some studies have documented fungal-induced SA accumulation under abiotic stresses, we compared SA accumulation in soybean upon two endophytic fungal interactions with host plants grown under saline conditions (Fig. 3). Plants exposed to salt stress showed a significantly lower concentration of SA

(1.10  $\mu\text{g/g}$  fwt) than controls (2.87  $\mu\text{g/g}$  fwt), but inoculation with endophytic fungi increased the SA level. The increase in SA content varied with *F. verticillioides* RK01 and *Humicola* sp. KNU01 treatments. *Humicola* sp. KNU01 fungi completely reversed the inhibition of SA level decrease in salt-stress-affected plants, while *F. verticillioides* RK01-associated plants showed a partially rescue of SA reduction induced by salinity. Khan et al. (2011b) also found that the endophytic fungus *Penicillium minioluteum* had the ability to enhance plant growth under salt stress conditions via increasing SA biosynthesis.

In conclusion, *F. verticillioides* RK01 and *Humicola* sp. KNU01 fungi are involved in effective stress avoidance in soybean plants grown in saline soils, as conformed by an increase of shoot length, protein, SOD activity and SA content, and a decrease in lipid peroxidation. Among these two fungal endophytes, *F. verticillioides* RK01 had a greater phosphate solubilization capacity than the *Humicola* sp. KNU01 isolate. The results of plant growth under stress conditions, together with the phosphate solubilization ability of *F. verticillioides* RK01, suggest that exogenous application of *F. verticillioides* RK01 was more favorable in enhancing soybean plant growth in saline environments compared to *Humicola* sp. KNU01 application.

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