Plant Physiology and Biochemistry 139 (2019) 459–469

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Cochliobolus sp. acts as a biochemical modulator to alleviate salinity stress in okra plants

Nusrat Bibi[a], Gul Jan[a], Farzana Gul Jan[a], Muhammad Hamayun[a], Amjad Iqbal[b,*], Anwar Hussain[a], Hazir Rehman[c], Abdul Tawab[d], Faiza Khushdil[a]

[a] Department of Botany, Garden Campus, Abdul Wali Khan University, Mardan, Pakistan
[b] Department of Agriculture, Garden Campus, Abdul Wali Khan University, Mardan, Pakistan
[c] Department of Microbiology, Garden Campus, Abdul Wali Khan University, Mardan, Pakistan
[d] National Institute of Biotechnology & Genetic Engineering, Jhang Road, Faisalabad, Pakistan

ARTICLE INFO

Keywords:
Salinity tolerance
Endophytic fungi
Cochliobolus lunatus
Okra plants
Physicochemical attributes

ABSTRACT

Salinity stress can severely affect the growth and production of the crop plants. Cheap and reliable actions are needed to enable the crop plants to grow normal under saline conditions. Modification at the molecular level to produce resistant cultivars is one of the promising, yet highly expensive techniques, whereas application of endophytes on the other hand are very cheap. In this regard, the role of *Cochliobolus* sp. in alleviating NaCl-induced stress in okra has been investigated. The growth and biomass yield, relative water content, chlorophyll content and IAA were decreased, whereas malondialdehyde (MDA) and proline content were increased in okra plants treated with 100 mM NaCl. On the contrary, okra plants inoculated with *C. lunatus* had higher shoot length, root length, plant dry weight, chlorophyll, carotenoids, xanthophyll, phenolicss, flavonoids, IAA, total soluble sugar and relative water content, while lower MDA. LC-MS/MS analysis of the *Cochliobolus* sp. extract revealed the presence of pinocembrin, chlorogenic acids, wogonin, calycosin and diadzein as a salinity stress reliever. From the results, it can be concluded that colonization of *Cochliobolus* sp. improves the NaCl tolerance by ameliorating the physicochemical attributes of the host plants.

1. Introduction

Plant microbe relations are among the significant processes, which are important for the existence of both the partners and sustainable agricultural system. The fungal endophytes also promote plant growth in diverse ways, i.e. by releasing plant growth regulators, enhancing mycorrhizal colonization and providing biologically fixed nitrogen (Bibi et al., 2018; Rai et al., 2014). Plant growth regulating micro-organisms are diverse class that is related to plants in different manners. This growth promoting effect by fungal endophyte can be due to the production of phytohormones, such as cytokines, indole acetic acid, or gibberellins (Bilal et al., 2018a; Ikram et al., 2018; Mehmood et al., 2018; Hamayun et al., 2017; Hussain et al., 2015). These plant hormones or secondary metabolites can help the host plants to thrive during biotic and abiotic stresses (Hamayun et al., 2015; Iqbal et al., 2018). In this interaction the interrelating partners is neither affected and the individual benefits rely on both the interacting associates (Zuccaro et al., 2011). Plant growth enhancement endophytic interaction has been observed useful to host plants even in stress environments (Ismail et al., 2018). Fungal endophytes have generally been investigated for their behavior to improve plants development because they influence main features of plant physiology and host defense against various biotic and abiotic stresses (Ismail et al., 2018; Mehmood et al., 2019). Salinity is one of the important abiotic stresses that limit plants growth, development and improvement in several areas of the world and leads to excessive reduction in plant production (Hamayun et al., 2017). Salinity stress is among the severe environmental stresses because it decreases crop production of more than 20% of irrigated land worldwide. Plants that grow in salty conditions are exposed to three different physiological stresses. The first toxic effects of sodium and chloride ions, prevailing in saline soils, interfere with the structure of enzymes and other macromolecules, interrupt respiration and photosynthesis, damage cell organelles, induce ion insufficiencies and prevent protein synthesis (Juniper and Abbott, 1993). Secondly plants in saline soils is subjected to low osmotic potential having great risk of physiological drought as they necessarily inhibit water movement from the roots to soil in order to retain lower internal osmotic potentials (Deinlein et al., 2014). Finally, saline soil also makes nutrient

* Corresponding author.
E-mail address: amjadiqbal@awkum.edu.pk (A. Iqbal).

https://doi.org/10.1016/j.plaphy.2019.04.019
Received 16 January 2019; Received in revised form 17 March 2019; Accepted 13 April 2019
Available online 14 April 2019
0981-9428/

imbalance in the plants by reducing the nutrient uptake (Ruiz-Lozano et al., 2012). Inside the plants, reactive oxygen species produces due to such stressful condition that may lead to cell death. During such stressful conditions, reactive oxygen species (superoxide, singlet oxygen, hydrogen peroxide and hydroxyl radical) donate the free electrons leaked from electron transport chains in chloroplasts and mitochondria (Ashraf and Harris, 2004; Zahid et al., 2016).

To develop and then cultivate salt-tolerant plants are the utmost challenge for the scientific community. Such practices are very expensive and time consuming, so cheap and quick alternative might be the best solution. In the last few years, it is presumed that endophytes might be a cost effective and sustainable way to alleviate salt stress in economically significant crops like okra (*Ablemoschus esculentus* L). Okra is a nutritionally significant summer vegetable that belongs to family Malvaceae. Salt stress causes negative effects on the development and crop production of okra. Proposed study, therefore, aimed to study the (i) adverse effects of salt stress on growth and crop production of okra plants (ii) role of endophytic fungi in alleviating salt stress (iii) regulation of phyto-hormones and secondary metabolites by endophytic fungi in conferring tolerance against salt stress.

2. Materials and methods

2.1. Plant material and fungal isolation

Different parts of *Mirabilis jalapa* L. were collect from the study site to isolate the plant growth promoting endophytic fungi. For that purpose, the plant parts were carefully washed with running tap water to eradicate any dust material stick to them. For the removal of any adhering microbes, the washed samples were surface sterilized with 70% ethanol for 30 s and 5% sodium hypochlorite for 5 min. The plant parts were then rinsed with double distilled water to remove sodium hypochlorite and ethanol. The wet parts were dried between the sterile filter paper and cut into small segments with sterilized blade. Four to five sterilized segments were carefully cultured in a petridish containing Hagem minimal medium having antibiotic streptomycin to arrest bacterial growth. Petridishes were wrapped with parafilm and kept for 15 days under dark at 25 °C and checked every day for fungal growth. Fungal isolates from Hagem minimal medium were purified on PDA plates incubated at 25 °C for 7 days (Hallmann et al., 2006).

2.2. Extraction of DNA from the isolated strains MJ1

Extraction of DNA from the selected strain was performed, according to well established protocol of Khan et al. (2008). The extracted DNA was then amplified by PCR. The purity of the extracted DNA and its quantity was measured by Thermo Scientific Nano Drop spectrophotometer at 260 nm (Chen and Kuo, 1993).

2.3. Identification of fungal isolate MJ1

Selected endophytic fungal strain was identified by amplifying their ITS region of 18S rDNA with universal primers, ITS-1 (5′-TCC GTA GGT GAA CCT GCGG-3′) and ITS-4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) (Lee Taylor and Bruns, 1999). A 20 ng of gDNA as tempelete was mixed with a 30 µl of EF-Taq (SolGent, Korea) and the mixture was placed in a PCR machine. The conditions of PCR were: 95 °C for 2 min; 35 cycles (95 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min); 72 °C for 10 min. The PCR products along with DNA markers (DNA ladder) were then loaded onto an agarose gel and subjected to electrophoresis for 30 min. The gel was developed by using 0.01 g/mL ethidium bromide stain and examined under UV lamp.

2.4. Sequencing of isolated MJ1

A purified PCR products of 1600 bp was sequenced with 18S rDNA region by utilizing universal primers ITS-1 (5′-TCC GTA GGT GAA CCT GCGG-3′) and ITS-4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) (Lee Taylor and Bruns, 1999). A Big Dye terminator cycle sequencing kit v.3.1 was used for that purpose. Both PCR sequencing and amplification was analysed by an automated DNA sequencing system (Applied Biosystems, Foster City, USA) at the Macrogen, Inc., Seoul, Korea.

The obtained PCR product was initially sequenced and then subjected to a homology search by using online tool, BLAST (http://blast.ncbi.nlm.nih.gov/Blast).

2.5. IAA production by MJ1

Endophytic fungi were grown in 50 mL Czapek broth at 30 °C and 120 rpm for 7 days to check IAA production. After 7 days of fungal incubation, culture filtrate of fungal isolates was subjected to UV spectrophotometer (530 nm) using Salkowski reagent to analyse IAA (Chadha et al., 2015).

2.6. Ammonia production by MJ1

Fungal isolate CGF-11 was evaluated for ammonia production in peptone water. Fungal cultures were transferred to glass tubes containing 10 mL of peptone water. The tubes were then placed in preset (27 °C) incubator for 48–72 h. After incubation, each tube were added with 0.5 mL of Nessler's reagent. The appearance of brown-yellow color upon the addition of Nessler's reagent indicated a positive test for ammonia production (Joseph et al., 2012).

2.7. Phosphate solubilization by MJ1

For the estimation of phosphate solubilization, endophytic fungal isolate was inoculated on the surface of Pikovskaya's solid agar medium in a petridish. After one to five days of incubation at room temperature, phosphate solubilizing activity was checked. Development of halo zones around fungal colony was a strong indication of phosphate solubilizing activity (Chadha et al., 2015).

2.8. Hydrogen cyanide (HCN) production by MJ1

Qualitative analysis of HCN production was determined as stated by Lorck (1948). Endophytic fungal isolate were cultured on PDA medium supplemented with glycine (4.4 g) per litter. Whatman filter paper was soaked in 2% Na_2CO_3 and picric acid (0.5%) solution and placed in the Petri plates. The plates were then wrapped with parafilm and were incubated at 27 °C for 7 days. Changing the color of Whatman filter paper from yellow to orange brown was an indication of cyanide production.

2.9. Estimation of phenolics in culture filtrate (CF) of MJ1

Phenolics in the CF of the isolated endophyte was estimated by the method of Qawasmeh, Raman, Wheatley and Nicol (Qawasmeh et al., 2012). A blue coloration indicates the presence of phenol and their absorbance was checked at 650 nm using SHIMADZU spectrophotometer (Kyoto, Japan). Gallic acid was used as a standard to quantify the total phenol in CF of fungal cultural filterate.

2.10. Estimation of flavonoids in CF of MJ1

Flavonoid in CF of isolated endophyte was estimated by the aluminum chloride colorimetric procedure as stated by Akbay et al. (2003). Absorbance was checked at 415 nm. Appearance of milky white color was a strong indication of flavonoid presence. Using quercetin calibration curve for flavonoids quantification was prepared.

2.11. Halo tolerance of the isolated MJ1

Halo tolerance of isolated fungal endophyte was checked by growing the fungal isolates in varying concentrations of salt (NaCl). Intially, the fungal endophyte was grown in Czapek broth medium supplemented with varying concentration of NaCl (50, 100 and 150 mM). Flasks were then incubated for 7 days at 27 °C and 120 rpm in shaking incubator. After 7 days, the fungal mycelia was filtered out and its fresh weight and dry weight was determined. Czapek broth medium without NaCl stress was used as a control (Hamayun et al., 2017).

2.12. Pot experiments for plant growth promotion activity by MJ1

Seeds of okra plant were surface sterilized by dipping them in 0.1% HgCl2 followed by washing (3-times) with double distilled water. The sterilized seeds were then soaked overnight in autoclaved distilled water. The soaked seeds were shifted to petri dishes (20 seeds/Petri dish) having two layers of autoclaved filter papers and were incubated for 3 days at 27 °C to germinate. Uniform germinated seedling were selected and transferred to the pots (10 seedlings/pot) having autoclaved soil.

T_1 = Control (-ive NaCl; -ive endophyte).
T_2 = Control (100 NaCl; -ive endophyte).
T_3 = MJ1 (-ive NaCl; 10 mL endophyte spore suspension).
T_4 = MJ1 + NaCl (100 NaCl; 10 mL endophyte spore suspension).

After the establishment of okra seedlings in pots, 10 mL of the fungal spore suspension was poured in to T_3 and T_4 pots. The seedlings were allowed to grow in pots for about two weeks under normal conditions to establish fungal colonization of okra plants. After two weeks of fungal colonization, the pots in T_2 and T_4 were supplemented with 100 mM NaCl. At the interval of three days, 10 mL of NaCl (100 mM) solution was applied to the plants from T_2 and T_4 treatments. On the 21st day of salt stress, the growth attributes (fresh, dry weight and root, shoot length) of harvested okra plants were measured.

2.13. Estimation of okra growth parameters

Root and shoot length was measured manually with the help of a scale. Plant fresh weight was measured directly using analytical balance, while plant dry weight was estimated after drying the samples in an oven for 48 h at 70 °C.

2.14. Determination of photosynthetic pigments in okra leaves

Contents of Chl a, Chl b, and carotenoids in the extract of fully expanded okra leaves before harvest was determined using MacKinney equations (Sestak, 1971). Fully expanded fresh leaves of fungal inoculated and non-inoculated maize plants under salt stress were homogenized with 2 mL of acetone (80%) and washed twice to reach final volume of 7 ml. The absorbance was measured using a spectrophotometer at 480 nm, 645 nm, and 663 nm.

2.15. Determination of relative water content (RWC) in okra

The procedure of Khan, Waqas, Hamayun, Al-Harrasi, Al-Rawahi and Lee (Khan et al., 2013) was adopted for the estimation of RWC.

$$\% RWC = \frac{FW - DW}{TW - DW} \times 100$$

2.16. Quantification of malondialdehyde (MDA) in okra

MDA was estimated by measuring malondialdehyde (MDA) content as stated by Najafabad and Jamei (2014). The optical density (OD) of supernatant was checked at 532 nm and 600 nm. Thiobarbituric acid TBA (0.25%) in (10%) trichloracetic acid TCA served as the blank.

2.17. Estimation of proline contents in okra

The procedure of Bagheri, Saadatmand, Niknam, Nejadsatari and Babaeizad (Bagheri et al., 2013) was used for proline determination in the leaves. Absorbance was checked at 520 nm by using toluene as a blank.

2.18. Estimation of sugar contents in okra

Sugar content was estimated by following the procedure of Zhang, Gan and Xu (Zhang et al., 2016) and optical density (OD) was measured at 490 nm by using UV Spectrophotometer. Ethanol (80%) was used as a blank.

2.19. Estimation of total phenolics and flavonoids in okra

Folin-Ciocalteau reagent method of Gurupavithra and Jayachitra (2013) was used for estimation of total phenolics content. Optical density of the supernatant was checked at 650 nm by using 80% ethanol as a blank. Flavonoid was estimated by aluminum chloride colorimetric procedure as given by Zhishen, Mengcheng and Jianming (Zhishen et al., 1999). The optical density was measured at 415 nm with a spectrophotometer.

2.20. Estimation of IAA in okra

Quantitative estimation of IAA in leaves was determined by Salkowski reagent as described by Zhishen, Mengcheng and Jianming (Zhishen et al., 1999). UV absorbance was checked at 540 nm by using UV spectrophotometer.

2.21. Statistical analysis

For statistical analysis, One-way analysis of variance (ANOVA) at P = 0.05 was used, followed by Duncan's Multiple Range Test (DMRT) by using SPSS for windows.

3. Results

3.1. Identification and phylogenetic analysis of bioactive endophyte MJ1

In the dendrogram, the fungal isolate MJ1 formed a sub-clade with a strain of DQ337381.1 *Cochliobolus lunatus* (97% bootstrap support). We aligned the ITS sequences of available *Cochliobolus lunatus* through the BLAST sequence, using Finch TV and Codon Code Aligner, and a neighbor joining tree was constructed from 17 algined sequences. The fungal isolate MJ1 formed a clade (97% bootstrap support) with monoclade of strains from *Cochliobolus lunatus* (550 bootstrap replications). The results suggested MJ1 as a new strain of *Cochliobolus lunatus* (Fig. 1). The sequence was submitted to the gene bank under accession number MK611790.

3.2. Plant growth promoting attributes of C. lunatus MJ1

The isolated strain was tested for their plant growth promoting attributes, such as phosphate solubilization, HCN and ammonia production. The present result showed that the isolated strain MJ1 were positive for HCN, phosphate solubilization and ammonia production (Table 1, Fig. S1).

3.3. Salt resistance by C. lunatus MJ1

The results revealed that the growth of isolated fungal strain *C. lunatus* MJ1 was unaffected in the presence of 50 and 100 mM NaCl. However, when NCl concentration was increased to 150 mM, the secretion of IAA, phenolics and flavonoids by fungal strain MJ1 were

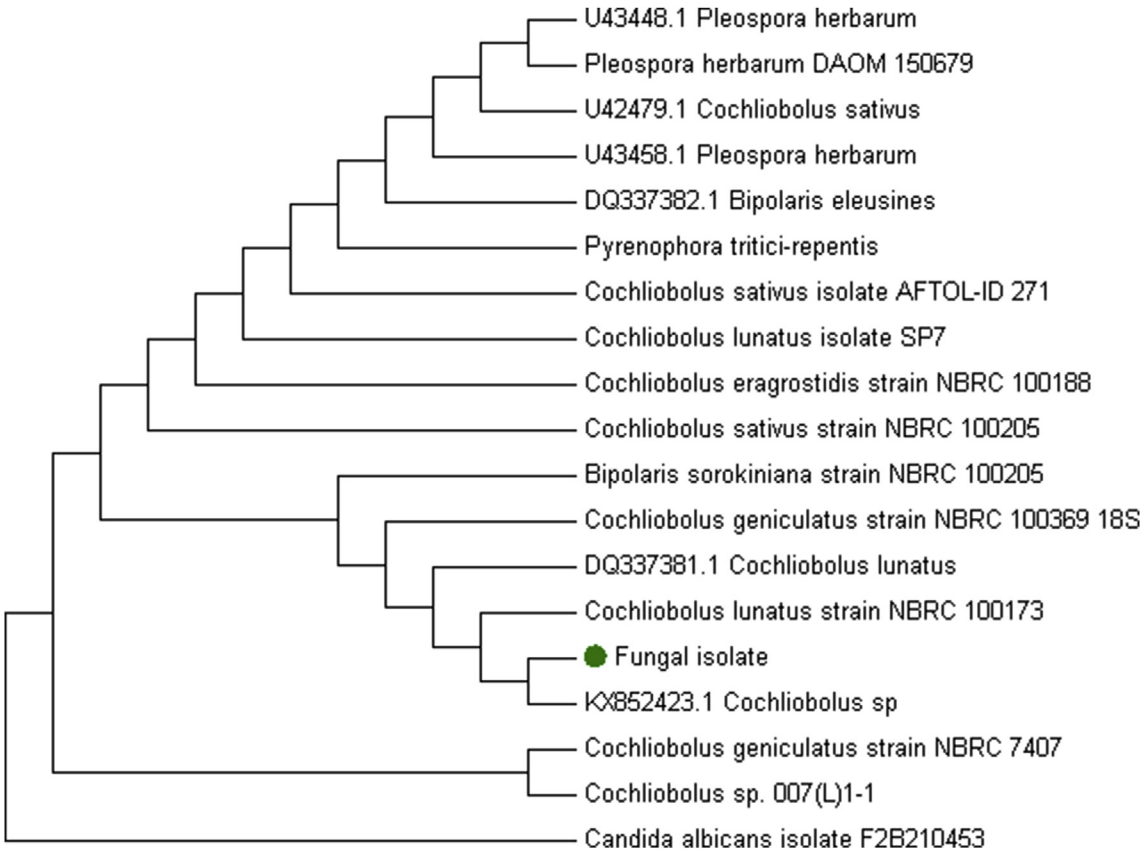

Fig. 1. Identification of endophytic fungal isolate MJ1 by phylogenetic analysis of the ITS region of 18S rDNA gene.

Table 1
Ammonia, HCN production and phosphate solubilization by fungal strain MJ1.

FS	Ammonia Production	HCN Production	Phosphate Solubilization
MJ1	+	+	+

FS = fungal strain; + = shows presence of activity.

significantly decreased (Fig. 2).

3.4. *C. lunatus* enhanced growth and biomass in NaCl supplemented okra plants

NaCl stress caused a decline in leaf number, shoot and root lengths, fresh and dry weights of okra plants (Figs. 3 and 4). Inoculation of these NaCl stressed okra plants with *C. lunatus* mitigated the salt stress by restoring all of the above parameters to the level of control. The okra plants when inoculated with *C. lunatus* have significantly (P = 0.05) more leaves than in control (Fig. 3A). The root and shoot length in *C. lunatus* associated okra seedlings were 29 cm and 15 cm that were longer than the control (Fig. 3Band C). Similarly the fresh and dry weights of NaCl stressed okra plant were 3 g and 0.5 g at 100 mM NaCl concentration relative to 4 g and 0.6 g in control. Fresh weight and dry weight of okra plants was improved by 34.03% and 30.55%, when inoculated with *C. lunatus* and supplemented with NaCl (Fig. 4Aand B).

3.5. *C. lunatus* induced stress resistance in okra plants

Results relating to the effect of salt stress and *C. lunatus* supplementation on the relative water content (RWC), total chlorophyll, proline and MDA are shown in Fig. 5. A decrease of 25.90% was observed in RWC at 100 mM NaCl concentration relative to the control plants. However, inoculation of okra plants with *C. lunatus* has increased the RWC to 24.83% under 100 mM NaCl stress (Fig. 5A). Moroevr, salt stress of 100 mM concentration yielded a decline in chlorophyll content by 52.91%, whereas *C. lunatus* associated okra plants subjected to salt stress showed significant (P = 0.05) increase in the chlorophyll contents (Fig. 5B). Proline contents were also improved in *C. lunatus* associated okra plants as compared to non-associated okra plants under saline conditions. An increase of 29.48% in proline contents was noticed in okra plants at 100 mM salt concentration, while maximum proline contents were perceived in okra plants inoculated with *C. lunatus* and NaCl stressed (Fig. 5C). Malondialdehyde (MDA) showed a maximum increase of 74.05% at 100 mM salt concentration (NaCl) relative to control plants. Decline in Malondialdehyde content was noticed in okra plants treated with *C. lunatus*. A reduction of 19.55% was observed in *C. lunatus* associated okra plants supplemented with 100 mM NaCl (Fig. 5D).

3.6. *C. lunatus* improved soluble sugar in okra plants grown under salt stress

C. lunatus has influenced the soluble sugar in okra plants grown in saline or non-saline conditions (Fig. 6). The results indicated a 36% increase in sugar contents, when okra plants were inoculated with fungal strains *C. lunatus* alone. However an increase of 15% was noted, when the *C. lunatus* inoculated okra plants undergone 100 mM NaCl stress. Soluble sugar contents in okra plants, markedly reduced when they were subjected to 100 mM salt stress without any association with *C. lunatus* (Fig. 6).

3.7. *C. lunatus* enhanced the level of phytoharmones and secondary metabolites in okra plants

Salt stress reduced IAA that showed a maximum reduction of 32.57%, 22.01% and 19.65%, respectively in the leaves, stem and roots

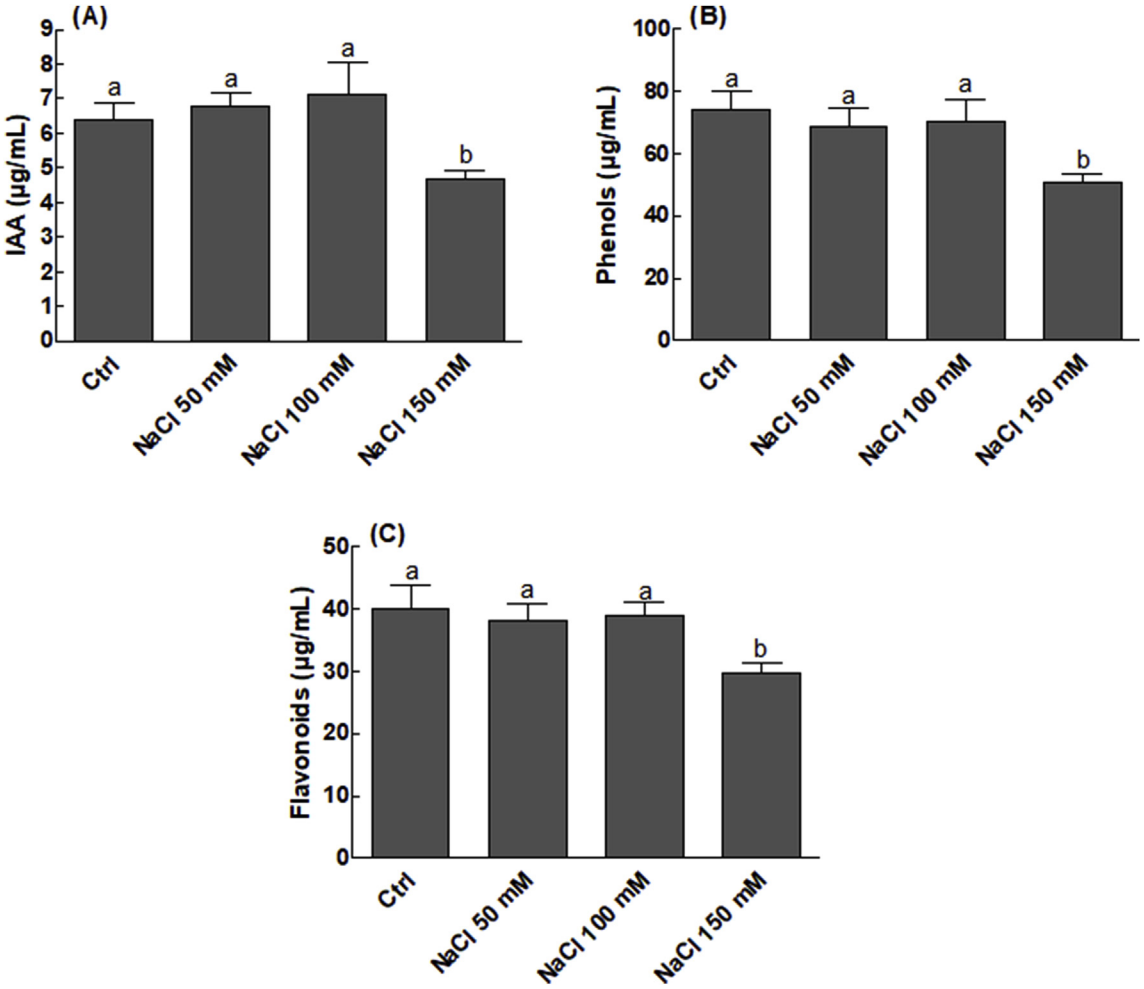

Fig. 2. Effect of different salt concentrations (NaCl) on IAA, phenolics and flavonoids production by *C. lunatus*. Ctrl = control; Fig. 2A represents IAA; Fig. 2B represents phenolics; Fig. 2C represents flavonoids in the culture filtrate of *C. lunatus* exposed to 50, 100 and 150 mM of NaCl. Each bar represents the mean of triplicated data with ± SE. Bars that are labeled with different letters are significantly different from one another at $p < 0.05$.

at 100 mM NaCl stress (Fig. 7A). Conversely, okra plants inoculated with *C. lunatus* indicated an increase of IAA in leaves (160.07%), stem (107.83%) and root (81.20%) when exposed to 100 mM of NaCl (Fig. 7A).

Phenolic and flavonoid contents were also enhanced by *C. lunatus*, but further increase was observed when the inoculated plants were subjected to 100 mM NaCl stress (Fig. 7Band C). The maximum values for phenolics and flavonoids were recorded in leaf (38 µg/mL and 37 µg/mL) of *C. lunatus* inoculated okra plants imperiled to salt stress. The amounts of total phenolics in leaf, stem and root of the *C. lunatus* associated okra plants exposed to 100 mM NaCl were increased by 29.31%, 27.68% and 18.71%, respectively (Fig. 7B). Likewise, an increase in flavonoids was noticed in okra plants colonized by the *C. lunatus*. The flavonoid contents of the plants were further enhanced in leaf (20.29%), stem (16.95%) and root (13.84%) of the *C. lunatus* colonized okra plants exposed to 100 mM NaCl stress (Fig. 7C).

3.8. Identification of compounds by LC MS/MS

The identification of the active compounds in *C. lunatus* extract was based on the LC- ESI-MS/MS spectral data and its comparison standards data. (Table 2). The compound 1 with *t* R = 6.83 showed [M-H] -ive ion at 255 *m/z* w, where its MS 2 spectrum showed fragment ions at 213 *m/z*. The resulted spectral data were compared with the previous standard data and the compound 1 was identified as pinocembrin (Table 2; Fig. S2). The LC MS/MS data of the compound 2 showed a precursor ion at 353 m/z with *t* R = 7.31. The data were consistent with the structure of the chlorogenic acid and thus the compound was classified as chlorogenic acid (Table 2; Fig. S3). Compound 3 with *t* R = 4.42 showed [M-H] -ive ion at 283.17 *m/z* a their MS 2 spectrum presented fragment ions at 163 *m/z*. The resulted data were coherent to the structure of wogonin, when compared with the reference standard and literature (Table 2; Fig. S4). Compound 4 (*t* R = 4.40) showed [M-H] -ive ion at 283.17 *m/z* with MS 2 spectrum presented fragment ions at 211.08 *m/z*. After comparing the spectral data of the compound 4 with a reference standard and literature the compound 4 was recognized calycosin (Table 2; Fig. S5). Furthermore, the LC MS/MS data of compound 5 showed a precursor ion at 353.08 m/z with *t* R = 2.43. The MS 2 spectrum of the compound 5 presented a fragment ions at 210.92 *m/z*. After the comparison of the ESI–MS/MS data with that of standards and as described in literature, the compound was identified as a diadzein (Table 2; Fig. S6).

4. Discussion

The present study was focused on the isolation of endophytic fungal strain that can be a halotolerent and act as a growth promoter. From mesophytic plant *Mirabilis jalapa* L. 12 endophytic fungal strains were isolated and tested against the okra plants under salt stress. About 10 of 12 endophytic fungal strains were found to be growth promoters, while 2 strains were growth inhibitors. Among the 10 growth promoting strains, MJ1 fungal strain has significantly increased shoot and root

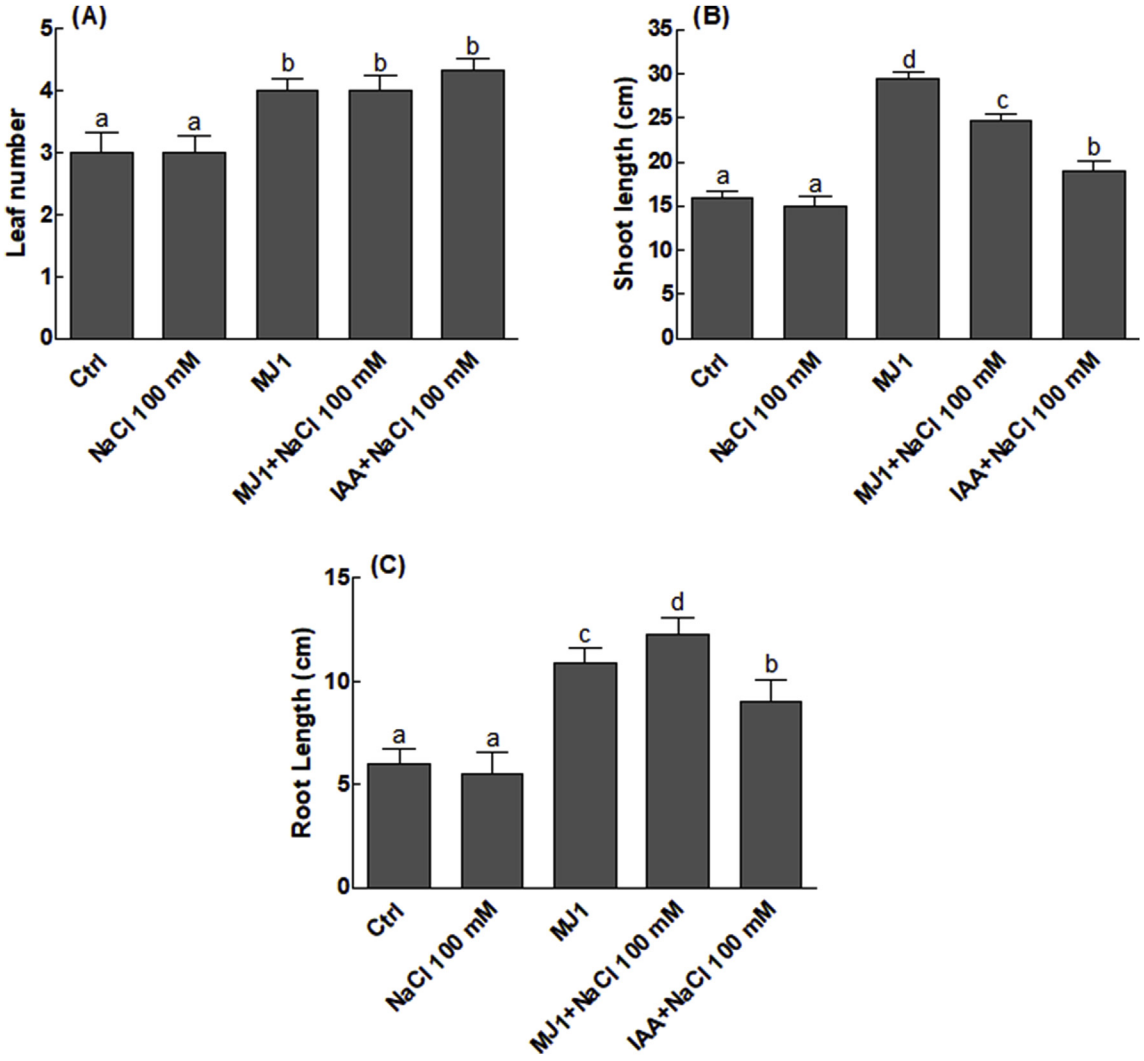

Fig. 3. Effect of NaCl stress on growth parameters of okra plants with or without *C. lunatus* association. Ctrl = control; Fig. 3A represents leaf number; Fig. 2B represents shoot length; Fig. 2C represents root lengths of *C. lunatus* associated and non-associated okra plants under 100 mM NaCl stress. Each bar represents the mean of triplicated data with ± SE. Bars that are labeled with different letters are significantly different from one another at $p < 0.05$.

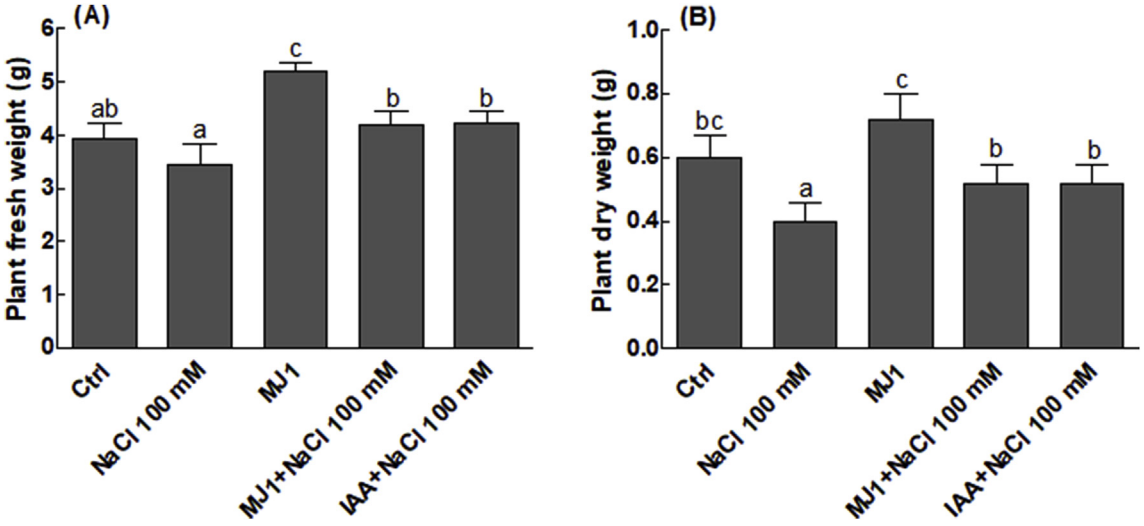

Fig. 4. Effect of NaCl stress on fresh and dry weight of okra plants with or without *C. lunatus* association. Ctrl = control; Fig. 4A represents the plant fresh weight; Fig. 4B represents plant dry weight of *C. lunatus* associated and non-associated okra plants under 100 mM NaCl stress. Each bar represents the mean of triplicated data with ± SE. Bars that are labeled with different letters are significantly different from one another at $p < 0.05$.

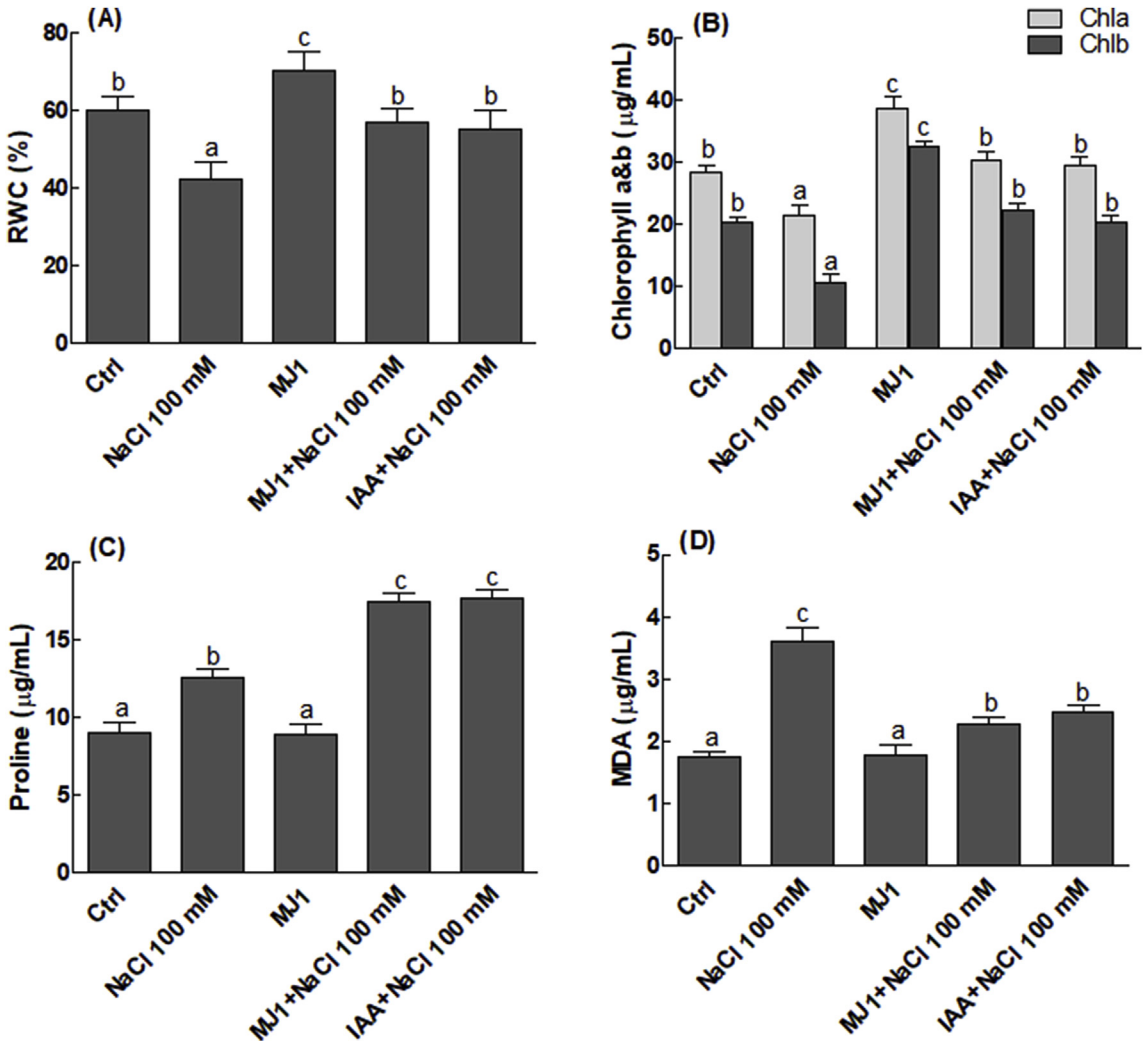

Fig. 5. Effect of NaCl on stress markers of okra plants with or without *C. lunatus* association. Ctrl = control; RWC = relative water contents; MDA = malondialdehyde; Fig. 5A represents RWC; Fig. 5B represents Chlorophyll *a*&b; Fig. 5C represents proline; Fig. 5D represents MDA of *C. lunatus* associated and non-associated okra plants under 100 mM NaCl stress. Each bar represents the mean of triplicated data with ± SE. Bars that are labeled with different letters are significantly different from one another at $p < 0.05$.

lengths up to 70.41% and 36.73%, in comparison to the control. While fresh and dry weight of the inoculated okra plant was also increased. These results are in accordance with the finding of Kedar, Rathod, Yadav, Agarkar and Rai (Kedar et al., 2014), who reported that endophytic *Phoma* species have the ability to boost the shoot length, root length, fresh weight and dry weight of inoculated maize plants significantly. Zhang, Gan and Xu (Zhang et al., 2016) also noticed a significant increase in wheat growth after association with the endophytic fungus *Trichoderma longibrachiatum*. The growth promotion in endophyte assisted plant species might be associated with the production of plant hormones, such as indole acetic acid (IAA) and gibbrellins (GA) (Ikram et al., 2018; Hamayun et al., 2017). The present study revealed that all the isolated growth promoting fungal strains have secreted IAA, but maximum IAA was secreted by MJ1. Moreover, the plant flavonoids and phenolics were increased by 99.95% and 57,70% after the inoculation of MJ1. Similar observations concerning the high production of flavonoids and phenolics have been recorded in maize plant inoculated with endophytic fungi (Kedar et al., 2014). Besides, the isolated MJ1 was positive for ammonia production, phosphate solubilization and hydrogen cyanide production (HCN), which is very common in plant growth promoting endophytes (Bibi et al., 2018; Bilal et al., 2018b). The potential of the isolated MJ1 strain as a halotolerant was checked by growing the strain in Czapek broth medium supplemented with varying concentration of NaCl (50 mM, 100 mM and 150 mM). In a NaCl supplemented medium (50 mM and 100 mM), the fresh and dry weight of MJ1 biomass was not affected significantly by the NaCl application, which shows MJ1 potential to resist salt stress. However when salt concentration was increased to 150 mM, the fresh and dry weight of fungal biomass was significantly reduced by 16%. Likewise, endophytic fungus *Porostereum spadiceum* AGH786 have shown similar results (Hamayun et al., 2017), which further confirms the present results. In the salt supplemented medium maximum Indole acetic acid (IAA) was produced by fungal strain MJ1. Increase in the IAA content of MJ1 was 2 folds as compared to non-stressed control at 100 mM NaCl concentration. Percent increase in flavonoids and phenolics content of MJ1 was 50.88% and 63.64%, respectively, as compared to fungal strain grown in control medium. The results of increasing flavonoids and phenolics content with NaCl stress are in accordance with the previous report (Sarwat et al., 2016).

4.1. C. lunatus enhanced growth and biomass yield in NaCl treated okra plants

Plant growth and biomass can drastically reduce under salinity stress that may be due to the non-availability of nutrients. The present study also indicated that the 100 mM salinity stress significantly

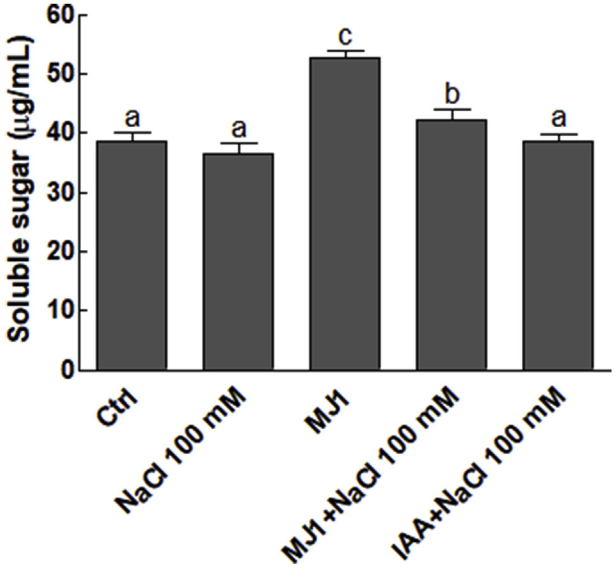

Fig. 6. Effect of NaCl stress on on sugar contents of okra plants with or without *C. lunatus* association. Ctrl = control. Each bar represents the mean of triplicated data with ± SE. Bars that are labeled with different letters are significantly different from one another at $p < 0.05$.

reduced all the growth parameters of okra plants. However the inoculation of NaCl stressed okra plants with *C. lunatus* mitigated the salt stress by restoring all growth parameters back to normal. Comparably, when soybean plants uder salinity were inoculated with endophytes a significant increase was observed in root and shoot growth (Khan et al., 2011), which supports the present data. The endophytic fungi secrete phytohormones, such as IAA and GAs that might be a reason for improved plant growth under salinity stress conditions.

4.2. *C. lunatus improved relative water content (RWC), total chlorophyll and proline content in NaCl treated okra plants*

The present study found that salinity stress drastically reduced the chlorophyll contents in okra plants, but with the inoculation of *C. lunatus*, the chlorophyll contents were reversed back to the level of control plants. Zhang, Gan and Xu (Zhang et al., 2016) also shown that salinity stress severely reduced the chlorophyll contents resulting in overall growth inhibition, but inoculation of plants with PGPF enhanced the chlorophyll. The possible reason for the decrease level of chlorophyll contents in okra plants under salinity was due to the alteration in mechanisms of (oxygen evolving complex) and chl-related proteins (Alqarawi et al., 2014). Carotenoids + xanthophyll contents of okra plants were greatly reduced at 100 mM NaCl stress. While carotenoid + xanthophyll contents was increased when okra plants were inoculated with fungal strain *C. lunatus*. Similar results have been documented by Ahmad, Hashem, Abd-Allah, Alqarawi, John, Egamberdieva and Gucel (Ahmad et al., 2015) that salinity stress decreased the carotenoids contents that recovered after the application of endophytes. A possible reason for enhanced pigment contents in okra plants was due to the synthesis of plant hormones, which stimulate the accumulation of carotenoids and chlorophyll. Increase in chlorophyll and carotenoids contents with *C. lunatus* inoculation may be due to inhibition of (Na) uptake. Moreover, proline an osmoprotectant was greatly increased in okra plant inoculated with fungal strain *C. lunatus* under saline conditions. Our results are in close agreement with the result of Khan, Hamayun, Radhakrishnan, Waqas, Kang, Kim, Shin, Choo, Kim and Lee (Khan et al., 2012), who observed an increase in proline contents in endophyte inoculated cucumber plant under saline condition. Greater accumulation of proline contents in *C. lunatus* inoculated okra plants might keep high leaf water potential under stress, thus protected them from oxidative damages.

4.3. *C. lunatus reduces lipid peroxidation in NaCl-treated okra plants*

Malondialdehyde (MDA) is a major lipid breakdown product and is a marker for oxidative damages. In the present results, the MDA level was increased significantly when okra plants were subjected to 100 mM NaCl. The increased level of MDA is observed by higher (ROS) production and lipid membrane damages. However, when okra plants were inoculated with fungal strain *C. lunatus* a reduced level of lipid peroxidation was recorded. This reduction in lipid peroxidation might lower cellular membrane damages in okra plants under salinity. These results are in accordance with that of Sarwat, Hashem, Ahanger, Abd_Allah, Alqarawi, Alyemeni, Ahmad and Gucel (Sarwat et al., 2016).

4.4. *C. lunatus enhanced the total flavonoids, phenolics and phytohormones in okra plants under NaCl stress*

Phenolics and flavonoids are non-enzymatic antioxidants that scavenge toxic radicals, and involved in plant defense against stress (Sarwat et al., 2016). Higher phenolic contents were noticed in leaf, stem and root of okra plants when subjected to 100 mM NaCl. However, further increase was observed when salt stressed okra plants were inoculated with fungal strain *C. lunatus*. The increase in phenolic contents improve the negative effects of NaCl stress in okra plants, which is alo supported by the results of Wada, Mizuuchi, Koshio, Kaneko, Mitsui and Takeno (Wada et al., 2014). During the LC MS/MS observation of this study, the compound that gave a precursor ion at 353 m/z was identified as chlorogenic acid. Chlorogenic acids are naturally occurring phenolic compound found in all higher plants. Like other natural compounds, phenolics help plants to adopt the changing environmental conditions, thus vital for plant defense mechanisms (Caretto et al., 2015).

Further, NaCl-stressed okra plants showed improved aggregation of flavonoids. Plants aggregating higher levels of flavonoids content revealed better NaCl tolerance as compared to the less flavonoid accumulating plants (Wahid and Ghazanfar, 2006). The flavonoid contents were increased in leaf, stem and roots of okra plants under saline conditions. However, when NaCl stressed okra plants were inoculated with *C. lunatus*, a further increase in flavonoids were observed. Similar results have been documented by Sarwat, Hashem, Ahanger, Abd_Allah, Alqarawi, Alyemeni, Ahmad and Gucel (Sarwat et al., 2016) in barley. The LC MS/MS analysis in the present study revealed various compounds. The compound having [M-H] -ive ion at 255 *m/z* and their MS 2 spectrum gave fragment ions at 213 *m/z* that was further identified as pinocembrin. Pinocembrin is a flavonoid constituent in numerous plants that has antifungal and antibacterial activity (Rasul et al., 2013). Moreover, Ahn, Kunimasa, Kumazawa, Nakayama, Kaji, Uto, Hori, Nagasawa and Ohta (Ahn et al., 2009) reported that pinocembrin has very low antioxidant activity, but possesses a significant degree of antiangiogenic activity. Another compound showed [M-H] ive ion at 283.17 *m/z* and fragment ions at 163 *m/z*, which was identified as wogonin. Wogonin is one of the major flavonoids. Wogonin has been generally investigated for its antioxidant, anticancer and anti-inflammatory activities. With increasing evidence of its anticancer therapeutic potential and striking toxicological properties, wogonin has been recognized as a capable lead compound for new anticancer drug development (Wu et al., 2016). The compound 4 was identified as calycosin, which exhibited [M-H] -ive ion at 283.17 *m/z* and its MS 2 spectrum presented fragment ions at 211.08 *m/z*. Clycosin as a flavone have demonstrated many pharmacological activities, such as antigenotoxic, antioxidant, anti-inflammatory, neuroprotective antinociceptive and anti-osteosarcoma functions (Li et al., 2016). Flavonoids usually accumulate as an aplastic response to biotic or abiotic stresses. Flavonoids suppress ROS (reactive oxygen species) levels in guard cells and thus modulate the dynamics of stomatal aperture

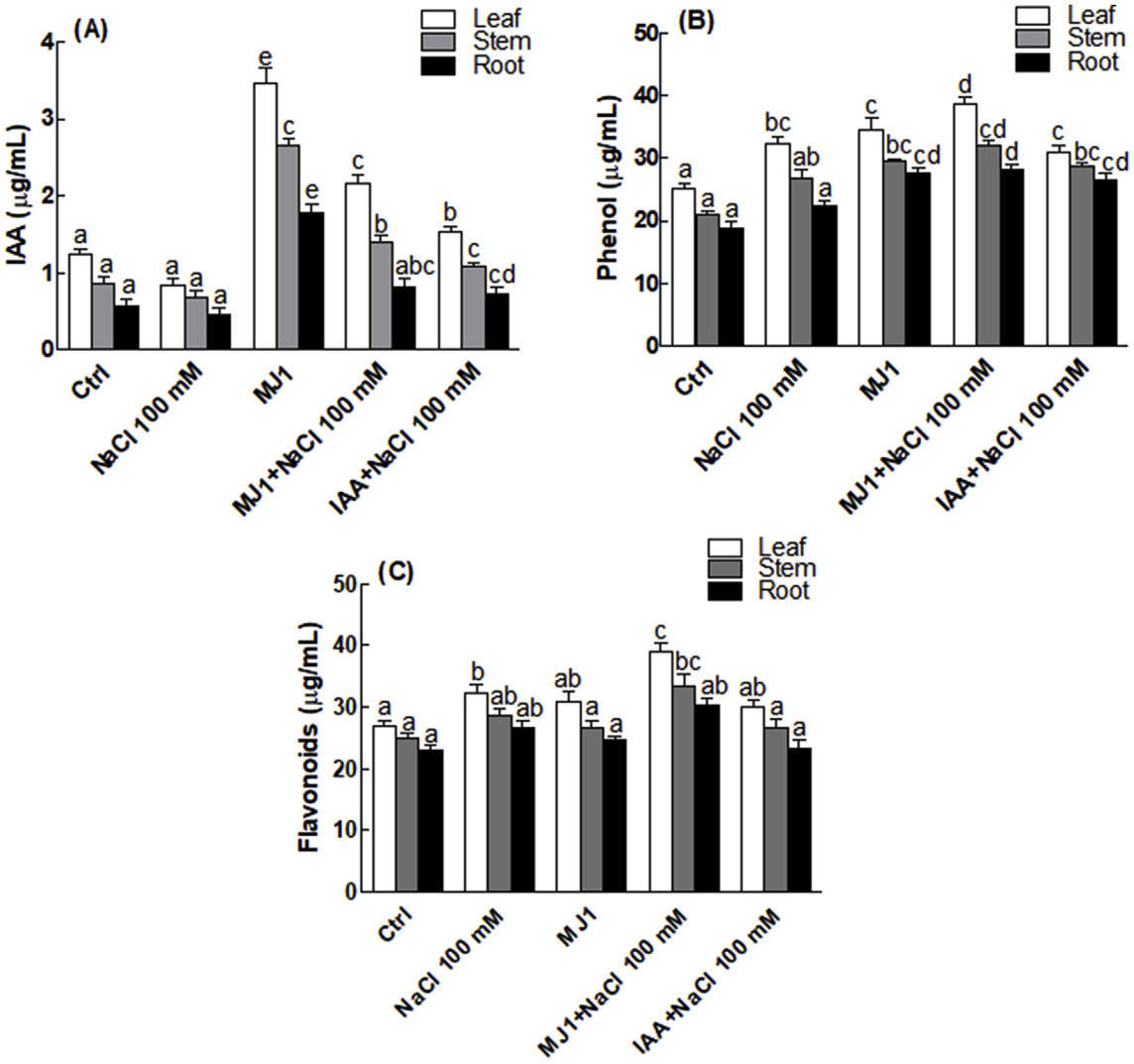

Fig. 7. Effect of NaCl stress on IAA, phenolics and flavonoids of okra plants with or without *C. lunatus* association. Ctrl = control; Fig. 7A represents IAA; Fig. 7B represents phenolics; Fig. 7C represents flavonoids of *C. lunatus* associated and non-associated okra plants under 100 mM NaCl stress. Each bar represents the mean of triplicated data with ± SE. Bars that are labeled with different letters are significantly different from one another at $p < 0.05$.

(Watkins et al., 2014). The final compound was identified as daidzein that gave a precursor ion at 353.08 m/z and MS 2 spectrum gave fragment ions at 210.92 *m/z*. Daidzein is one of the most prominent soy isoflavones, is largely restricted to leguminous plants. The health benefits and versatile pharmacological properties of daidzein including its anti-cancer, anti-osteoporosis, anti-cardiovascular disease, anti-diabetic, anti-oxidant, anti-aging, and anti-inflammatory activities have been widely investigated. Furthermore, daidzein is also stated to exhibit various bio-activities against dermatosis and neurodegenerative diseases (Zhao et al., 2018).

Also, the okra plants under NaCl stress have reduced IAA contents. Nonetheless, when okra plants were inoculated with *C. lunatus*, an increase in IAA was noted. Such increase in phenolic and flavonoid contents, and a decrease in IAA contents under NaCl stress was also examined by Sarwat, Hashem, Ahanger, Abd_Allah, Alqarawi, Alyemeni, Ahmad and Gucel (Sarwat et al., 2016).

4.5. Improved conents of soluble sugar in C. lunatus associated okra plants

The soluble sugar has been widely known as an important indicator in abiotic stress responses (de Azevedo Neto et al., 2006). The increased accumulation of sugar content in *C. lunatus* associated okra plants under saline conditions might protected the oxidative damages at a cellular level. Zhang, Gan and Xu (Zhang et al., 2016) also found that endophytic fungi has significantly increased the sugar content in wheat seedlings grown under saline or non-saline environment.

Table 2
Characterization of compounds in culture filtrate of *C. lunatus* by LC/MS MS.

NO	*t*R (min)	Precursor Ion, *m/z*	Major Fragment Ions	Identification
1	6.83	[M-H] −255.00	255.00, 237.08, 213.00, 192.83, 186.00, 140.92	Pinocembrin
2	7.31	[M-H] −253.25	353.25, 177.00, 163.00	Chlorogenic acid
3	4.42	[M-H] −383.17	383.17, 265.33, 241.00, 223.17, 163.00, 106.92	Wogonin
4	4.40	[M-H] −283.17	283.17, 265.33, 241.00, 223.17, 211.08, 183.08, 163.00, 106.92	Calycosin
5	2.43	[M-H] −253.08	253.08, 210.92, 191.00, 183.93, 173.08	Diadzein

Funding statement

Abdul Wali Khan University Mardan has financially supported the project.

Ethics approval and consent to participate

Our study doesn't involve any human, animal or endangered species.

Consent for publication

No consent/approval at the national or international level or appropriate permissions and/or licenses for the study was required.

Availability of data and material

All the data are included in the manuscript.

Conflicts of interest

The authors declare that there is no competing interest of any nature related to this manuscript.

Author's contribution

NB, FK, GJ and FGJ designed and performed all the experiments. HR and AT performed the LC/MS/MS analysis. AI and AH analysed and wrote the manuscript. AI and MH edited the manuscript and arranged the resources for the work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.04.019.

References

Ahmad, P., Hashem, A., Abd-Allah, E.F., Alqarawi, A., John, R., Egamberdieva, D., Gucel, S., 2015. Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (*Brassica juncea* L) through antioxidative defense system. Front. Plant Sci. 6, 868.

Ahn, M.R., Kunimasa, K., Kumazawa, S., Nakayama, T., Kaji, K., Uto, Y., Hori, H., Nagasawa, H., Ohta, T., 2009. Correlation between antiangiogenic activity and antioxidant activity of various components from propolis. Mol. Nutr. Food Res. 53, 643–651.

Akbay, P., Basaran, A.A., Undeger, U., Basaran, N., 2003. In vitro immunomodulatory activity of flavonoid glycosides from Urtica dioica L. Phytother Res. 17, 34–37.

Alqarawi, A.A., Abd Allah, E., Hashem, A., 2014. Alleviation of salt-induced adverse impact via mycorrhizal fungi in *Ephedra aphylla* Forssk. J. Plant Interact. 9, 802–810.

Ashraf, M., Harris, P., 2004. Potential biochemical indicators of salinity tolerance in plants. Plant Sci. 166, 3–16.

Bagheri, A.A., Saadatmand, S., Niknam, V., Nejadsatari, T., Babaeizad, V., 2013. Effect of endophytic fungus, *Piriformospora indica*, on growth and activity of antioxidant enzymes of rice (*Oryza sativa* L.) under salinity stress. Int. J. Adv. Biol. Biomed. Res. 1, 1337–1350.

Bibi, S., Hussain, A., Hamayun, M., Rahman, H., Iqbal, A., Shah, M., Irshad, M., Qasim, M., Islam, B., 2018. Bioremediation of hexavalent chromium by endophytic fungi; safe and improved production of *Lactuca sativa* L. Chemosphere 211, 653–663.

Bilal, L., Asaf, S., Hamayun, M., Gul, H., Iqbal, A., Ullah, I., Lee, I.-J., Hussain, A., 2018a. Plant growth promoting endophytic fungi *Asprgillus fumigatus* TS1 and *Fusarium proliferatum* BRL1 produce gibberellins and regulates plant endogenous hormones. Symbiosis 1–11.

Bilal, L., Asaf, S., Hamayun, M., Gul, H., Iqbal, A., Ullah, I., Lee, I.-J., Hussain, A., 2018b. Plant growth promoting endophytic fungi *Asprgillus fumigatus* TS1 and *Fusarium proliferatum* BRL1 produce gibberellins and regulates plant endogenous hormones. Symbiosis 76, 117–127.

Caretto, S., Linsalata, V., Colella, G., Mita, G., Lattanzio, V., 2015. Carbon fluxes between primary metabolism and phenolic pathway in plant tissues under stress. Int. J. Mol. Sci. 16, 26378–26394.

Chadha, N., Prasad, R., Varma, A., 2015. Plant promoting activities of fungal endophytes associated with tomato roots from central Himalaya, India and their interaction with *Piriformospora indica*. Int. J. Pharm. Biol. Sci. 6, 333–343.

Chen, W.-p., Kuo, T.-T., 1993. A simple and rapid method for the preparation of gram-negative bacterial genomic DNA. Nucleic Acids Res. 21, 2260.

de Azevedo Neto, A.D., Prisco, J.T., Enéas-Filho, J., de Abreu, C.E.B., Gomes-Filho, E., 2006. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. Environ. Exp. Bot. 56, 87–94.

Deinlein, U., Stephan, A.B., Horie, T., Luo, W., Xu, G., Schroeder, J.I., 2014. Plant salt-tolerance mechanisms. Trends Plant Sci. 19, 371–379.

Gurupavithra, S., Jayachitra, A., 2013. Isolation and identification of endophytic fungi from *Ocimum sanctum* and analyse its antioxidant properties. Int. J. Res. Pharmaceut. Biomed. Sci. 4, 1120–1125.

Hallmann, J., Berg, G., Schulz, B., 2006. Isolation procedures for endophytic microorganisms. In: Microbial Root Endophytes. Springer, pp. 299–319.

Hamayun, M., Hussain, A., Khan, S.A., Irshad, M., Khan, A.L., Waqas, M., Shahzad, R., Iqbal, A., Ullah, N., Rehman, G., 2015. Kinetin modulates physio-hormonal attributes and isoflavone contents of Soybean grown under salinity stress. Front. Plant Sci. 6, 377.

Hamayun, M., Hussain, A., Khan, S.A., Kim, H.-Y., Khan, A.L., Waqas, M., Irshad, M., Iqbal, A., Rehman, G., Jan, S., 2017. Gibberellins producing endophytic fungus *Porostereum spadiceum* AGH786 rescues growth of salt affected soybean. Front. Microbiol. 8, 686.

Hussain, A., Shah, S.T., Rahman, H., Irshad, M., Iqbal, A., 2015. Effect of IAA on in vitro growth and colonization of Nostoc in plant roots. Front. Plant Sci. 6, 46.

Ikram, M., Ali, N., Jan, G., Jan, F.G., Rahman, I.U., Iqbal, A., Hamayun, M., 2018. IAA producing fungal endophyte Penicillium roqueforti Thom., enhances stress tolerance and nutrients uptake in wheat plants grown on heavy metal contaminated soils. PLoS One 13, e0208150.

Iqbal, A., Shah, F., Jamal, Y., Hamayun, M., Islam, B., Khan, Z.H., Hussain, A., Rehman, G., Ziaullah, Shah, S., 2018. Detection of food allergens by ELISA and other common methods. Fresenius Environ. Bull. 8340.

Ismail, Hamayun, M., Hussain, A., Iqbal, A., Khan, S.A., Lee, I.-J., 2018. Endophytic fungus *Aspergillus japonicus* mediates host plant growth under normal and heat stress conditions. BioMed Res. Int. 2018.

Joseph, B., Ranjan Patra, R., Lawrence, R., 2012. Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L.). Int. J. Plant Prod. 1, 141–152.

Juniper, S., Abbott, L., 1993. Vesicular-arbuscular mycorrhizas and soil salinity. Mycorrhiza 4, 45–57.

Kedar, A., Rathod, D., Yadav, A., Agarkar, G., Rai, M., 2014. Endophytic Phoma sp. isolated from medicinal plants promote the growth of *Zea mays*. Nusantara Biosci. 6, 132–139.

Khan, S.A., Hamayun, M., Yoon, H., Kim, H.-Y., Suh, S.-J., Hwang, S.-K., Kim, J.-M., Lee, I.-J., Choo, Y.-S., Yoon, U.-H., 2008. Plant growth promotion and *Penicillium citrinum*. BMC Microbiol. 8, 231.

Khan, A.L., Hamayun, M., Kim, Y.-H., Kang, S.-M., Lee, I.-J., 2011. Ameliorative symbiosis of endophyte (*Penicillium funiculosum* LHL06) under salt stress elevated plant growth of Glycine max L. Plant Physiol. Biochem. 49, 852–861.

Khan, A.L., Hamayun, M., Radhakrishnan, R., Waqas, M., Kang, S.-M., Kim, Y.-H., Shin, J.-H., Choo, Y.-S., Kim, J.-G., Lee, I.-J., 2012. Mutualistic association of *Paecilomyces formosus* LHL10 offers thermotolerance to *Cucumis sativus*. Antonie Leeuwenhoek 101, 267–279.

Khan, A.L., Waqas, M., Hamayun, M., Al-Harrasi, A., Al-Rawahi, A., Lee, I.-J., 2013. Co-synergism of endophyte Penicillium resedanum LK6 with salicylic acid helped *Capsicum annuum* in biomass recovery and osmotic stress mitigation. BMC Microbiol. 13, 51.

Lee Taylor, D., Bruns, T.D., 1999. Population, habitat and genetic correlates of mycorrhizal specialization in the 'cheating'orchids *Corallorhiza maculata* and *C. mertensiana*. Mol. Ecol. 8, 1719–1732.

Li, Y., Wang, C., Li, H., Yu, T., Tan, L., 2016. Simultaneous determination of formononetin, calycosin and rhamnocitrin from *Astragalus complanatus* by UHPLC–MS-MS in rat plasma: application to a pharmacokinetic study. J. Chromatogr. Sci. 54, 1605–1612.

Lorck, H., 1948. Production of hydrocyanic acid by bacteria. Physiol. Plantarum 1, 142–146.

Mehmood, A., Hussain, A., Irshad, M., Hamayun, M., Iqbal, A., Khan, N., 2018. In vitro production of IAA by endophytic fungus *Aspergillus awamori* and its growth promoting activities in Zea mays. Symbiosis 1–11.

Mehmood, A., Hussain, A., Irshad, M., Hamayun, M., Iqbal, A., Rahman, H., Tawab, A., Ahmad, A., Ayaz, S., 2019. Cinnamic acid as an inhibitor of growth, flavonoids exudation and endophytic fungus colonization in maize root. Plant Physiol. Biochem. 135, 61–68.

Najafabad, A.M., Jamei, R., 2014. Free radical scavenging capacity and antioxidant activity of methanolic and ethanolic extracts of plum (*Prunus domestica* L.) in both fresh and dried samples. Avicenna J. Phytomed. 4, 343.

Qawasmeh, A., Raman, A., Wheatley, W., Nicol, H., 2012. Antioxidative capacity of phenolic compounds extracted from *Lolium perenne* and *Lolium arundinaceum* infected with Neotyphodium (Hypocreales: clavicipitaceae). Acta Physiol. Plant. 34, 827–833.

Rai, M., Rathod, D., Agarkar, G., Dar, M., Brestic, M., Pastore, G.M., Junior, M.R.M., 2014. Fungal growth promotor endophytes: a pragmatic approach towards sustainable food and agriculture. Symbiosis 62, 63–79.

Rasul, A., Millimouno, F.M., Ali Eltayb, W., Ali, M., Li, J., Li, X., 2013. Pinocembrin: a novel natural compound with versatile pharmacological and biological activities. BioMed Res. Int. 2013.

Ruiz-Lozano, J.M., Porcel, R., Azcón, C., Aroca, R., 2012. Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: new challenges in physiological and molecular studies. J. Exp. Bot. 63, 4033–4044.

Sarwat, M., Hashem, A., Ahanger, M.A., Abd_Allah, E.F., Alqarawi, A., Alyemeni, M.N., Ahmad, P., Gucel, S., 2016. Mitigation of NaCl stress by arbuscular mycorrhizal fungi

through the modulation of osmolytes, antioxidants and secondary metabolites in mustard (*Brassica juncea* L.) plants. Front. Plant Sci. 7.
Sestak, Z., 1971. Determination of chlorophylls a and b, Plant photosynthetic production. Manual Methods 673–701.
Wada, K.C., Mizuuchi, K., Koshio, A., Kaneko, K., Mitsui, T., Takeno, K., 2014. Stress enhances the gene expression and enzyme activity of phenylalanine ammonia-lyase and the endogenous content of salicylic acid to induce flowering in pharbitis. J. Plant Physiol. 171, 895–902.
Wahid, A., Ghazanfar, A., 2006. Possible involvement of some secondary metabolites in salt tolerance of sugarcane. J. Plant Physiol. 163, 723–730.
Watkins, J.M., Hechler, P.J., Muday, G.K., 2014. Ethylene-induced flavonol accumulation in guard cells suppresses reactive oxygen species and moderates stomatal aperture. Plant Physiol. 164, 1707–1717.
Wu, X., Zhang, H., Salmani, J.M.M., Fu, R., Chen, B., 2016. Advances of wogonin, an extract from Scutellaria baicalensis, for the treatment of multiple tumors. OncoTargets Ther. 9, 2935.
Zahid, K.R., Ali, F., Shah, F., Younas, M., Shah, T., Shahwar, D., Hassan, W., Ahmad, Z., Qi, C., Lu, Y., 2016. Response and tolerance mechanism of cotton *Gossypium hirsutum* L. to elevated temperature stress: a review. Front. Plant Sci. 7, 937.
Zhang, S., Gan, Y., Xu, B., 2016. Application of plant-growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. Front. Plant Sci. 7, 1405.
Zhao, W., Shang, Z., Li, Q., Huang, M., He, W., Wang, Z., Zhang, J., 2018. Rapid screening and identification of daidzein metabolites in rats based on UHPLC-LTQ-orbitrap mass spectrometry coupled with data-mining technologies. Molecules 23, 151.
Zhishen, J., Mengcheng, T., Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64, 555–559.
Zuccaro, A., Lahrmann, U., Güldener, U., Langen, G., Pfiffi, S., Biedenkopf, D., Wong, P., Samans, B., Grimm, C., Basiewicz, M., 2011. Endophytic life strategies decoded by genome and transcriptome analyses of the mutualistic root symbiont *Piriformospora indica*. PLoS Pathog. 7, e1002290.