Mycorrhizal fungi induced activation of tomato defense system mitigates Fusarium wilt stress

Abeer Hashema, Adnan Akhterb, Abdulaziz A. Alqarawic, Garima Singhd, Khalid F. Almutairic, Elsayed Fathi Abd_Allahc

a Botany and Microbiology Department, College of Science, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia
b Faculty of Agricultural Sciences, Department of Plant Pathology, University of the Punjab, Lahore, P.O Box: 54590, Pakistan
c Plant Production Department, College of Food and Agricultural Sciences, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia
d Department of Botany, Pachhunga University College (PUC), Aizawl 796001, Mizoram, India

A B S T R A C T

The fungus Fusarium oxysporum f. sp. lycopersici (FOL) is known to cause vascular wilt on tomato almost over the world. Inoculation of FOL reduced plant growth and increased wilt of tomato. The following study examined the possible role of arbuscular mycorrhizal fungi (AMF) consortium comprising of Rhizopogon intraradices, Funneliformis mosseae and Claroideoglomus etunicatum against FOL in tomato and explored in an inducing plant systemic defense. AMF inoculation reduced the wilt disease within vascular tissue and in vivo production of fusaric acid was observed which may be responsible in reduced wilting. FOL had an antagonistic effect on AMF colonization, reduced the number of spores, arbuscules and vesicles. AMF also inhibited the damage induced by Fusarium wilt through increasing chlorophyll contents along with the activity of phosphate metabolising enzymes (acid and alkaline phosphatases). Moreover, tomato plants with mycorrhizal inoculation showed an increase in the level of antioxidant enzymes including glutathione reductase, catalase, and etc. with an ultimate influence on the elimination of reactive oxygen species. Moreover, rise in phosphatase along with antioxidant enzymatic systems and enhanced photosynthetic performance contributed to induced resistance against FOL in tomato.

© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Tomato (Solanum lycopersicum L.) a member of the family Solanaceae, is an important food crop consumed worldwide. Tomato is rich in nutrients and anti-cancer and anti-oxidative compounds like lycopene and flavonoids (Gerszberg et al., 2015).

Cellular processes like photosynthesis, respiration, plasma membrane functions and water conductivity are affected by pathogenic fungi (Berger et al., 2007). This biotic stress leads towards the over accumulation of toxic reactive oxygen species (ROS) thus inducing oxidative stress in plant (Vellesillo et al., 2010). Excess accumulated ROS interacts with the cellular constituents including lipids, proteins and nucleic acids, thus hinders the normal working of the cell (El-Rahman et al., 2012; Egamberdieva et al., 2017; Hashem et al., 2017). One of the biotic stress induced by Fusarium oxysporum f. sp. lycopersici (FOL) is responsible for intense yield losses of tomato due to wilt disease (Nirmaladevi and Sirnivas 2012; Akhter et al., 2015).

To alleviate the adverse effect of wilt disease caused by FOL biologically, the biologists are looking for alternative means, arbuscular mycorrhizal fungi (AMF) one of the most effective biological strategy reported to control wilt diseases (Al-Hmoud and Al-Momany, 2015). AMF are ubiquitous and improves the plant growth and development via enhancing the nutrient uptake and the rhizospheric soil health (Nahiyam, and Matsubara 2012; Al-Hmoud and Al-Momany, 2015). AMF induced resistance by enhancing the accumulation of defense related proteins, osmolytes and strengthening of the antioxidant system (Alqarawi et al., 2014; Abd_Allah et al., 2015; Akhter et al., 2015). The antioxidant system constituted of the reactive and non-reactive components which can mediate the elimination of ROS, hence protect the plants from the stress induced by oxidative burst (Nahiyam and Matsubara, 2012; Egamberdieva et al., 2017). Superoxide dismutase (SOD),
catalase (CAT), ascorbate peroxidase (APX) and etc. (El-Rahman et al., 2012; Kurbachew and Wydra, 2014; Abd_Allah et al., 2015), exhibit a close coordination in neutralizing the ROS. AMF induced positive changes reflect as an improvement in growth of host plants and subsequently improve their potential to withstand the stress triggered deleterious changes (Al-Hmoud and Al-Momany 2015; Hashem et al., 2016). Amelioration of the stress induced by the AMF has also been observed by active involvement of key phytohormones like auxins, cytokines, jasmonates and etc. (Cao et al., 2011; Beneduzi et al., 2012; Denancé et al., 2013; Hashem et al., 2015; Petti et al. (2012) and Buhrow et al. (2016) have demonstrated that the up-regulated expression of genes encoding indole acetic acid, indole butyric acid, and nine-cis-epoxy carotenoid dioxygenase involved in the synthesis of abscisic acid improved tolerance to Fusarium head blight in barley. Therefore, symbiotic association between AMF and plants provides new avenues for developing alternative strategies against plant pathogenic fungi (Nahiyan and Matsubara, 2012; Lewadowski et al., 2013; Song et al., 2015).

Therefore, the present study was aimed to assess the influence of Fusarium oxysporum f. sp. lycopersici on the development of tomato and FOL induced wilt severity and the impact of AMF (Rhizophagus intraradices, Claroideoglomus etunicatum, Funneliformis mosseae) were observed in mitigating the disease severity via enhancing the antioxidant metabolism, phytohormones homeostasis and osmolytes accumulation.

2. Material and methods

2.1. Plant material

Certified tomato seeds (Solanum lycopersicum L., cv Red Rock) were treated with sodium hypochlorite (NaOCl, 5.0%, v/v) for 5 min to surface sterilize the seeds and washed afterwards with double distilled water. The seeds were sown in plastic plates (25x25x5 cm) containing autoclaved peat, perlite and sand (1:1:1, v/v/v) under controlled conditions (day/night temperature of 26/16 °C; Relative humidity, 56%) for two weeks after germination. The developed seedlings used for pathogenicity testing and pot experiments.

2.2. Fusarium oxysporum f. sp. Lycopersici isolation and inoculum preparation

Fusarium oxysporum f. sp. lycopersici (FOL) was isolated from tomato fields in Saleheyah Al Gidadah city (30.685725, 31.882915), Sharqia Governorate, Egypt (Fig. 1). Root tissue fragments of symptomatic tomato plants were surface-sterilized and plated on potato dextrose agar (PDA, Difco Laboratories, Detroit, MI, USA) amended with 50 mg L of antibiotic tetracycline. The inoculated PDA plates were incubated at 25 °C for 7–10 days under standard conditions according to Summerell et al. (2003), for the development mycelial growth of Fusarium oxysporum (Summerell et al., 2003) and sub-cultured onto PDA slants. The developed mycelia and conidia were characterized according to Booth (1977) and Nelson et al. (1983).

2.3. Disease incidence and severity assessment

Three weeks old healthy tomato seedlings were inoculated by standard root dip method as described by Nirmaladevi and Srinivas (2012). The tomato seedlings were gently uprooted. The root tip (about 1 cm) was slightly trimmed and immersed for 30 min in the conidial suspension (10⁶ CFU [colony forming units] ml⁻¹ with sterile deionized water) of phytopathogen (FOL), carboxymethyl cellulose (CMC, 0.05%, w/v) used as adhering agent. Seedlings dipped in sterile water with CMC served as control. Afterwards, the seedlings were transplanted to plastic pots (25 cm diameter), containing autoclaved soil and sand (1:1). Five seedlings per pot were transplanted. Tomato plants were placed in a greenhouse where temperatures range varied between 25 and 30 °C. The plantlets were watered two times per week (50 mL/pot) and fertilized once a week with NPK (15:15:15). Disease incidence was assessed after 6 weeks of inoculation. The disease index used throughout the experiments calculated as percentage according to the next equation:

Disease incidence (%) = Number of infected plants / Total number of plants × 100

The brownish discoloration of the xylem vessel (percent invaded vessels) was confirmed and measured by slitting the stem (Johnson et al., 1982).

2.4. Arbuscular mycorrhizal fungi (AMF) and its application

The endophytic AMF (Rhizophagus intraradices, Funneliformis mosseae, Claroideoglomus etunicatum), were isolated previously from Talh trees (Acaica gerrardii) roots grown natively in Khuraim Meadow in Riyadh, Saudi Arabia (Hashem et al. 2016) according to the protocol as narrated by Daniels and Skipper (1982) and modified by Uboho et al. (2011). The trap culture protocol of Stutz and Morton (1996) was followed in this study. The inoculum of AMF was added to each pot at the application rate of 25 g of trap culture (counting approx.100 spores/g trap culture)/pot. Pots without mycorrhiza served as the control.

2.5. Experimental design, treatments and plant growing conditions

Completely randomized design experiment with ten replicates (one plant/each pot) was laid out to study the effect of AMF on FOL in tomato. The treatments were given as follows:

(1): Control (Without FOL and AMF inoculation); (2): FOL only; (3): FOL + AMF; (4): AMF only. The pots were placed in growth chamber. The disease incidence was assessed after 6 weeks of inoculation, subsequently the plant samples were collected for analyses.

2.6. Photosynthetic pigments

Tomato leaves (100 mg) were first extracted in acetone, then absorbance was measured at 622, 645, and 470 nm on spectrophotometer (Lichtenthaler and Wellburn, 1983). Chlorophyll and carotenoids contents were estimated by following formulae

\[
\text{Chl a} = 117.5A_{662} - 2.35A_{645} \\
\text{Chl b} = 18.61A_{645} - 3.96A_{662} \\
C_{xc} = (1000A_{700} - 2.27C_{a} - 81.4C_{b})/227 \\
\]

where: Chl a: chlorophyll a contents; Chl b: chlorophyll b, and C_{xc}: carotenoids contents

2.7. Determination of leaf relative water content

Relative water contents (LRWC) of leaves were estimated by punching discs from the leaf of each treated plant. After calculating the fresh weight, the same leaf discs were kept on water for 4 h for the calculation of turgid weight. The leaf samples were dried in oven at 85 °C to obtain dry weight (Smart and Bilgham, 1974). Calculation of leaf water content was done by the following formula:
LRWC = \frac{\text{fresh weight of leaf}}{\text{turgid weight of leaf}} \times 100

2.8. Determination of antioxidant enzyme activities

Frozen leaf tissue (0.4 g) samples were homogenized in pre-chilled mortar and pestle using 4 mL ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 4% (w/v) polyvinyl pyrroldone. The mixture was centrifuged at 14000 rpm at 4 °C for 30 min and the supernatant was used as enzyme source. Superoxide dismutase (SOD, EC1.15.1.1) activity was determined according to the Beauchamp and Fridovich (1971). Ascorbate peroxidase (APX, EC1.11.1.1) activity was assayed by observing the change in absorbance at 290 nm. While, APX activity was calculated by using molar extinction coefficient (ε) of 2.8 mM⁻¹ cm⁻¹ for AsA and activity expressed as U mg⁻¹ protein (Nakano and Asada, 1981). For the measurement of dehydro ascorbate reductase (DHAR, EC: 1.8.5.1) activity, the method of Nakano and Asada (1981) was employed. Glutathione reductase (GR, EC1.6.4.2) activity was estimated by following the protocol of Smith et al. (1988).

2.9. Estimation of fusaric acid

Fusaric acid was estimated using thin-layer chromatography. Spots are developed on the chromatogram descending for 10–12 h in sec-butanol formic acid–water solvent system (75:15:10 v/v). The chromatograms were placed under hood (14–16 h) for drying and bromophenol blue was sprayed. Fusaric acid gives a yellow color (Stefan, 2005).

2.10. Estimation of AMF colonization

At the harvesting time, the AMF spores were isolated from the the soil substrate from every treatment by wet sieving and decanting method as described by Daniels and Skipper (1982) and modified by Utobo et al. (2011). The intensity of mycorrhizal colonization (mycelium, vesicles and arbuscules) was determined by the following formula:

\[
\text{AMF root colonization} (%) = \frac{\text{total no. of AMF positive segments}}{\text{total no. of segments studied}} \times 100
\]

2.11. Statistical analysis

The experimental data were analyzed by employing two-way analysis of variance (ANOVA) with the help of Statistical Analysis System (SAS version 9.1) software. Significant differences between means were calculated by the least significant differences (LSD) test at P = 0.05. Additionally, the correlation coefficients were calculated for the studied parameters.

3. Results

3.1. Influence of FOL and AMF on tomato growth parameters

The morphological growth parameters were significantly higher in AMF inoculated treatment in comparison to control treatment (not inoculated with FOL). AMF exhibited significant improvement in the growth with an increase in shoot and root length (30.07% and 26.29%, respectively). However, upon inoculation with FOL...
Effect of *Fusarium oxysporum* triggered wilt disease on the length (cm / plant) and dry weight (gm / plant) of shoot and root in *Solanum lycopersicum* with and without AMF inoculation. Data presented is mean of three replicates.

| Treatments         | Shoot height (cm) | Shoot dry wt (g) | Root depth (cm) | Root dry wt (g) | Shoot height/Root depth | Shoot / Root dry wt |
|--------------------|------------------|------------------|-----------------|-----------------|------------------------|---------------------|
| Control            | 29.86b           | 0.5093b          | 14.3b           | 0.257b          | 2.100b                 | 1.992b              |
| Fusarium Only      | 8.93d            | 0.1963d          | 5.53d           | 0.1133d         | 1.621c                 | 1.789c              |
| Fusarium + AMF     | 21.63c           | 0.3593c          | 9.6c            | 0.1776c         | 2.253a                 | 2.019a              |
| AMF Only           | 42.7a            | 0.706a           | 19.4a           | 0.3813a         | 2.201a                 | 1.859b              |
| LSD at 0.05:       | 4.78             | 0.066            | 1.642           | 0.036           | 0.42                   | 0.58                |

Table 2

Effect of *Fusarium oxysporum* triggered wilt disease on chlorophyll pigments (mg/ g fresh wt) and net photosynthetic rate (mmol CO2 M\(^{-2}\) S\(^{-1}\)) in *Solanum lycopersicum* with and without AMF inoculation. Data presented is mean of three replicates.

| Treatments         | Photosynthetic activity | Photosynthetic pigments (mg / g fresh wt) | Net photosynthetic rate |
|--------------------|-------------------------|-----------------------------------------|------------------------|
|                    |                         | Chl a | Chl b | Chl a + b | Chl a/b | Carotenoids | Total pigments | Photosynthetic activity |
| Control            |                         | 1.091b | 0.6973b | 1.788b | 1.564d | 0.3910d | 2.179b | 12.03b |
| Fusarium Only      |                         | 0.5003d | 0.1736d | 0.674d | 2.928a | 0.8026a | 1.476d | 4.58d  |
| Fusarium + AMF     |                         | 0.7953c | 0.4666c | 1.262c | 1.707c | 0.5906b | 1.852c | 8.15c  |
| AMF Only           |                         | 1.640a | 0.7990a | 2.439a | 2.053b | 0.4113c | 2.850a | 14.63a |
| LSD at 0.05:       |                         | 0.082  | 0.045  | 0.1073 | 0.492  | 0.022  | 0.12   | 1.24   |

3.2. Influence of FOL and AMF on photosynthetic pigments

Tomato plants inoculated with AMF only exhibited a significant improvement in pigment content. Relative to the control increase in chlorophyll \(a\), chlorophyll \(b\), carotenoids and total pigments was 33.47%, 12.73%, 4.93% and 23.54%, respectively (Table 7). Tomato infected with FOL exhibited a reduction of 54.14%, 75.10% and 32.60%, respectively) was observed upon inoculation with AMF. Fusarium infection reduced the dry shoot and root weight by 61.22% and 55.91%, respectively, however plants treated with both FOL and AMF together exhibited only 29.45% and 30.89% reduction (Table 6).

3.3. Influence of FOL and AMF on leaf relative water contents

Mycorrhizae inoculated tomatoes showed an increase of 6.83% in the leaf relative water content (LRWC) as compared to uninoculated plants, however FOL induced wilt resulted in 43.30% reduction in LRWC (Table 8). The plants received AMF inoculum exhibited significant increase in flavonoid content under infected free as well as diseased conditions. Relative to the control, an increase in total flavonoids was observed in AMF inoculated plants both without and with FOL (FOL + AMF) infected conditions (44.00% and 71.12%, respectively). However, FOL induced wilt resulted in 50.98% decline in LRWC (Table 8).

3.4. Influence of AMF on wilt development and accumulation of fusaric acid

FOL triggered wilt and its disease incidence potential in tomato was 85.22% and 90.51% as wilted plants and invaded vessels, respectively (Table 1). Moreover, the infection of tomato plants was accompanied with accumulation of fusaric acid (22.09 mg/ g root fresh weight) as wilt inducing agent. Inoculation tomato plant with the AMF, caused significant decrease in disease incidence (wilted plants and invaded vessels) and fusaric acid accumulation in roots (79.04% and 10.92%, respectively), compared to the plants without AMF (Table 1).

3.5. Determination of AMF root colonization and correlation with FOL development

Colonization and total spore number of AMF were lower significantly in FOL-inoculated plants (Table 2). FOL infection resulted in considerable reduction in the mycelia (39.43%), vesicles (55.85%) and arbuscules (52.14%) as well as total spore number of AM fungi (52.61%) compared to control mycorrhizal plants. The intensity of fungal infection (structural colonization) in tomato plants with M along the intensity of AMF as \(M\) and \(A\) were reduced in FOL plants significantly, however \(P\) had significantly higher intensity as compared non-diseased plants (Table 3). The intensity of AMF was always comparable to the infection of FOL. The Pearson’s cor-

Table 3

Effect of *Fusarium oxysporum* triggered wilt disease on leaf relative water content (%) in *Solanum lycopersicum* with and without AMF inoculation. Data presented is mean of three replicates.

| Treatments         | LRWC % |
|--------------------|--------|
| Control            | 86.09b |
| Fusarium Only      | 48.82d |
| Fusarium + AMF     | 64.14c |
| AMF Only           | 92.40a |
| LSD at 0.05        | 2.57   |
relation coefficient between colonization of AMF and disease incidence of tomato caused by FOL was presented in the Table 4. The wilted plants have positive but non-significant effect on invaded vessels (IV), (M) and (V) as (0.937), (0.343) and (0.343) respectively, while non-significant and negative correlation were recorded for fusaric acid and (A) as (-0.597) and (-0.985), respectively. Invaded vessels (IV) showed negative correlation with fusaric acid, (M), (V) and (A). Fusaric acid showed positive and non-significant correlation with A (0.726) while negative correlation was recorded for both M and V. Mycelium showed highly significant and positive correlation for tomato vesicles, while vesicles showed negative correlation for (A) (Table 4).

3.6. Influence of AMF on phosphatase and antioxidant enzyme activity

Mycorrhizal inoculation on tomato plants resulted in significant increase activities of both phosphatase enzymes (acid & alkaline) as compared to un-inoculated control plants (Fig. 2A, B). In contrast, FOL inoculation of tomato plants caused drastic decline in the activity of acid and alkaline phosphatases by 41.91% and 55.97%, respectively, as compared with the un-inoculated control plants. However, the pre-inoculation of tomato plants with AMF resulted in strong induction of acid and alkaline phosphatases compared with treatment only inoculated with FOL. The Pearson’s correlation coefficients between colonization and phosphatase enzymes is described in the Table 5. The mycelium showed non-significant but positive correlation against the vesicles (0.796) and arbuscules (0.752) while acid phosphatase (0.954) and alkaline phosphatase (0.965) recorded positive and significant correlation. On the other hand vesicles showed positive while non-significant correlation on arbuscules (0.932), ACP (0.786) and ALP (0.792). The arbuscules have positive and significant results on ACP (0.834) and ALP (0.824) respectively. ACP showed highly significant and positive correlation with Alkaline phosphatase activity (0.998). Tomato plants infected with FOL triggered an increase in SOD, APX, DHAR and GR activity. Tomato plants with FOL + AMF inoculation showed 51.75%, 12.75%, 18.83% and 26.38% increase in activity of SOD, APX, DHAR and GR, respectively (Fig. 3A-D).

| Treatments                  | Total spore number | Mycelium | Vesicles | Arbuscules |
|-----------------------------|--------------------|----------|----------|------------|
| Fusarium + AMF             | 664.6b             | 57.3b    | 20.0b    | 34.6b      |
| AMF Only                    | 1402.3a            | 94.6a    | 45.3a    | 72.3a      |
| LSD at 0.05:                | 352.118c           | 37.87    | 15.34    | 26.69      |

*Total spore number: spore per 250 g soil.

| Treatments                  | Mycelium (M) | Vesicles (V) | Arbuscules (A) |
|-----------------------------|--------------|--------------|----------------|
| Fusarium + AMF             | 71.0a        | 83.3a        | 83.3a          |
| AMF Only                    | 45.0b        | 63.0b        | 61.3b          |
| LSD at 0.05:                | 18.37        | 12.47        | 11.04          |
Fig. 3. A-D: Effect of *Fusarium oxysporum* triggered wilt disease on activity of (A) superoxide dismutase, (B) ascorbate peroxidase (C) dehydroascorbate reductase, (D) glutathione reductase with and without AMF in *Solanum lycopersicum* L. Data presented are the means ± SE (n = 5).
Table 7
Pearson Correlation Coefficients between Colonization and Disease incidence.

|   | WP | IV | FA | M  | V   | A    |
|---|----|----|----|----|-----|------|
| WP| 1.00000| 0.93731| -0.59780| 0.34370| 0.34370| -0.98512|
| IV| 0.2266| 0.5921| 0.7766| -0.00511| 0.7766| 0.1100|
| FA| 1.00000| -0.28095| 0.0817| 0.9967| 0.9967| 0.3366|
| M | 1.00000| 1.00000| -0.95827| 0.1846| 0.1846| 0.4821|
| V | 0.0578| 0.0845| 0.0666| 0.0845| 0.0666| -0.0001|
| A | 1.00000| 1.00000| 0.83415| 0.83415| 0.83415| 0.82407|

WP: Wilt plants; IV: invaded vessels; FA: Fusaric acid; M: Mycelium; V: Vesicles; A: Arbuscules.

Table 8
Pearson Correlation Coefficients between Colonization and Phosphatases enzymes.

|   | M  | V   | A    | ACP | ALP   |
|---|----|-----|------|-----|-------|
| M | 1.00000| 0.79676| 0.75227| 0.95467| 0.96594|
| V | 0.0578| 0.0845| 0.0666| 0.0030| 0.0017|
| A | 1.00000| 0.83415| 0.83415| 0.0390| 0.0437|
| ACP| 0.1846| 0.78697| 0.78697| 0.0959| 0.0959|
| ALP| 1.00000| 0.82407| 0.82407| 0.98984| 0.99884|

M: Mycelium; V: Vesicles; A: Arbuscules; ACP: Acid phosphatase; ALP: Alkaline phosphatase.

4. Discussion

Tomato showed drastically reduced growth in plants inoculated with FOL. The phytotoxic potential of FOL is related to production of fusaric acid as wilt inducing agent which played a major role in a significant decrease of plant growth via photosynthesis inhibition (Landa et al., 2002; Wu et al., 2008). Our study revealed that there is a substantial accumulation of fusaric acid in Fusarium infected plants compared with control. In another context, water deficit stress developed by FOL which caused blocking of vascular system in tomato roots hence, significantly enhanced restricting plant growth rate when only limited resource was available during stress (Lima et al., 2019). Increased growth and biomass production with AMF inoculation was observed in both FOL inoculated and uninoculated plants. Several reports including Al-Askar and Rashad (2010) Nahiyan, and Matsubara (2012) and Al-Hmoud and Al-Momany (2015) has reported enhanced growth of AMF inoculated plants in both healthy and diseased conditions on different crops. The induction of defense associated proteins including pathogenesis-related proteins (PRP) and cell wall degrading chitinase and β-1,3-glucanase by AMF are known to induce systemic resistance against Fusarium oxysporum (Pozo et al., 2010). Disease resistance in tomatoes could be due to improved growth conditions. However the mechanisms actually involved was not established. Production of PRP is considered an indicator of induced defense response while accumulation of chitinases and β-1,3-glucanase also linked with inducing resistance against Alternaria solani in tomato and AMF-colonization induced increase in growth of the host is mainly due to the increased nutrient acquisition particularly the phosphorous (Evelin et al., 2009; Beltrano et al., 2013; Huang et al., 2014; Hashem et al., 2015). The beneficial impact of associations between plant roots and AMF enhance uptake & mobility of nutrients like inorganic phosphate to host plants in exchange for fixed carbon source, food for AMF (Garcia et al., 2016; Bukovská et al., 2018). Phosphatases play their role in increasing the availability of phosphorous to plants (Liao et al., 2003). In our investigation, acid and alkaline phosphatases decreased with FOL infection, whereas AMF ameliorated the effect considerably. In accordance to our findings, Zhang et al. (2014) has also reported the reduced uptake of phosphorous in pea due to the reduction in phosphatase activity. Valliyodan et al. (2017) has demonstrated considerable enhancement in the activity of phosphate assimilating enzymes in soybean due to AMF. In our study root phosphatase activity was higher in mycorrhizae inoculated plants. Probably, the higher resistance of the existing acid phosphatase to the degradation by stress-induced enzyme as well as production of acid phosphatases were among the prime reasons for improved acid phosphatase activity (Jakobek and Lindgren 2002; Liao et al., 2003). Beltrano et al. (2013) and Zhang et al. (2014) advocates that improved root phosphatase activity regulates phosphorous transport and assimilation. Tomato plants infected with Fusarium wilt had shown reduced AMF colonization which may be due to the release of fusaric acid by FOL. Fusaric acid inhibit the growth of microflora either natural or beneficial to plants (Landa et al., 2002). Earlier Al-Askar and Rashad (2010) has demonstrated significant reduction in the AMF root colonization in beans due to Fusarium root rot disease. Similarly, Nahiyan and Matsubara (2012), Lewandowski et al. (2013) and Al-Hmoud and Al-Momany (2015) had also reported decline in the AMF root colonization in different crop plants infected with root pathogens. Moreover, fusaric acid produced by the F. oxysporum has the potential to inhibit photosynthesis and reduced chlorophyll synthesis (Wu et al., 2008), and it could have promoted the activity of chlorophyll degrading enzymes chlorophyllase concomitant with the decline in the Rubisco activase activity leading to reduced photosynthetic rate (Akhter et al., 2015). We also found significantly lower photosynthetic pigments in FOL-treated tomato plants. Our results are in line with the previous reports of other F. oxysporum attacks on watermelon (Wu et al., 2008); onion (Abdelrahman et al., 2016) and banana (Thakker et al., 2013). Moreover, the storn-
Al-Askar, A.A., Rashad, Y.M. 2010. Arbuscular mycorrhizal fungi: a biocontrol agent against common bean Fusarium root rot disease. Plant Pathol. J. 9 (1), 31–38.

Al-Hmoud, G., Al-Momany, A.J., 2015. Effect of four mycorrhizal products on Fusarium root rot on different vegetable crops. Pathol Plant Microb. 6, 2. https://doi.org/10.4172/2157-7471.1000255.

Alqarawi, A.A., Abd_Allah, E.F., Hashem, A. 2014. Alleviation of salt-induced adverse impact via mycorrhizal fungi in Ephedra aphylla Forsk. J. Plant Interact. 9 (1), 1002–1010. http://www.tandfonline.com/doi/full/10.4172/2157-7471.1000153.

Beaucamp, C., Fridovich, I., 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. Rev. 44, 276–287.

Beltzer, A., Casciani, M., Traini, D., Casati, A., Ruscitti, M., Tosti, M. 2013. Effects of arbuscular mycorrhiza inoculation on plant growth, biological and physiological parameters and mineral nutrition in pepper grown under different salinity and P levels. J. Soil Sci. Plant Nutr. 13 (1), 123–141.

Benbenishty, A., Ambrosini, A., Pasquala, L.M., 2015. Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. Genet. Mol. Biol. 35, 1044–1051. https://doi.org/10.1590/S1415-47572015000600020.

Berger, S., Sinha, A.K., Rotsch, T., 2007. Plant physiology meets phytophytophthora: plant primary metabolism and plant–pathogen interactions. J. Exp. Bot. 58 (15/16), 4019–4026. https://doi.org/10.1093/jxb/erm298.

Booth, C., 1977. Fusarium: Laboratory Guide Identification of the Major Species. Commonwealth Mycological Institute (CMI), Kew, England.

Buhrow, L.M., Cram, D., Tulpan, D., Foroud, N.A., Loewen, M.C., 2016. Exogenous asascic acid and gibberelicalic acid elicit opposing effects on Fusarium graminearum induction in wheat. Phytopathology 106 (9), 986–996. https://doi.org/10.1094/PHYTO-04-15-0140-R.

Bukovská, P., Bonkowski, M., Konvalinková, T., Beskid, O., Hujslová, M., Püschel, D., Rˇezácˇová, V., Gutiérrez-Núñez, M.S., Gryndler, M., Jansa, J., 2018. Utilization of organic nitrogen by arbuscular mycorrhizal fungi: is there a specific role for peptidases and ammonia oxidizers? Mycorrhiza 28, 269–283.

Cao, F.Y., Yoshio, K., Desveaux, D., 2015. The roles of ABA in plant–pathogen interactions. J. Plant Res. 124, 489–499. https://doi.org/10.1007/s10265-011-0409-4.

Daleke, B.A., Skipper, H.D., 1982. Methods for the recovery and quantitative estimation of propagules from soil. In: Schenck, N.C. (Ed.). Methods and Principles of Mycorrhizal Research, The American Phytopathological Society, pp. 29–36.

Delgadillo, R., Zakaria, L., Mohamad, A., Joniyas, A., Subramaniam, S., 2015. Effects of Fusaric acid treatment on the protocorm-like bodies of Dendrobium sonin-28. Prototplasma. https://doi.org/10.1007/s00709-015-0895-1.

Denaden, N., Sánchez-Vallés, A., Grofner, D., Molina, A., 2013. Disease resistance or growth: the role of plant hormones in balancing immune responses and fitness costs. Front Plant Sci. 2013 (4), 155. https://doi.org/10.3389/fpls.2013.00155.

Eganberdieva, D., Wirth, S.J., Shurigin, V.V., Hashem, A., Abd_Allah, E.F., 2017. Endophytic Bacteria improve plant growth, symbiotic performance of chickpea (Cicer arietinum L.) and induce suppression of root rot caused by Fusarium solani under salt stress. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.01887.

El-Raghy, T.H., Saad, A.A., Maizen, M.M., Mohamed, H., Mahno, N.M., 2012. Induction of defence related enzymes and phenolic compounds in lupin (Lupinus albus L.) and their effects on host resistance against Fusarium wilt. Eur. J. Plant Pathol. 134, 105–116. https://doi.org/10.1007/s10658-012-0028-z.

Evelin, J., Kapero, P., Girì, B., 2009. Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann. Bot. 104, 1263–1280. https://doi.org/10.1093/aob/mc251.

Garcia, K., Doidy, J., Zimmermann, S.D., Courty, P.E., 2016. Take a trip through the plant and fungal transportome of mycorrhiza. Trends Plant Sci. 21, 937–950.

Gerszberg, A., Hnatuszko-Konka, K., Kowalczyk, T., Kononowicz, A.K., 2015. Tomato (Solanum lycopersicum L.) in the service of biotechnology. Plant Cell Tiss. Organ. Cult. 120, 881–902.

Hashem, A., Abd_Allah, E.F., Alqarawi, A.A., Radhakrishnan, R., Kumar, A., 2017. Plant defense approach of Bacillus subtilis (BERA 71) against Macrophomina phaseolina (Tassi) Goid in mung bean. J. Plant Int. 12 (1), 390–401. https://doi.org/10.1080/17429145.2017.1373871.

Hashem, A., Abd_Allah, E.F., Ahmed, P., 2015. Effect of AM fungi on growth, physio-chemical attributes, lipid peroxidation, antioxidant enzymes and plant growth regulators in Lycopersicon esculentum Mill. subjected to different concentrations of NaCl. Pak. J. Bot. 47, 327–340.

Hashem, A., Abd_Allah, E.F., Alqarawi, A.A., Al-Huqail, A.A., Wirth, S., Eganberdieva, D., Shim et al. 2016. The interaction between arbuscular mycorrhizal fungi and endophytic bacteria enhances plants growth of Acacia gerrardi under salt stress. Front. Microbiol. 7, 1089. https://doi.org/10.3389/fmicb.2016.01089.

Huang, Y.M., Srivastava, A.K., Zou, Y.N., Ni, Q.D., Han, Y., Wu, Q.S., 2014. Mycorrhiza-induced calmodulin mediated changes in antioxidant enzymes and growth regulated enzymes of salt-stressed tomato plants. Front Microbiol. 5, 682. https://doi.org/10.3389/fmicb.2014.00682.

Jakobek, J.L., Lindgren, P.B. 2002. Expression of a bean acid phosphatase CDS is correlated with disease resistance. J. Exp. Botany 53, 367–389. https://doi.org/10.1093/jxb/erj367.

Johnson, L.E.B., Froshieierz, F.L., Wilcoxson, R.D., 1982. Interaction between Fusarium oxysporum f. sp. medicaginis and Corynebacterium insidiosum in alfalfa. Phytopathology 72, 517–522.

A. Hashem, A. Akhter, A.A. Alqarawi et al. - Al-Saudi Journal of Biological Sciences 28 (2021) 5442–5450

References

Abd_Allah, E.S., Hashem, A., Alqarawi, A.A., Alwathnani, H.A., 2015. Alleviation of adverse impact of cadmium stress in sunflower (Helianthus annuus L.) by arbuscular mycorrhizal fungi. Pak. J. Bot. 47 (2), 785–794.

Abdelrahman, M., Abdel-Motaal, F., El-Sayed, M., Jogaah, S., Shigo, M., Ito, S., Tran, L.S.P., 2016. Dissection of Trichoderma longibrachiatum-induced defense in onion (Allium cepa L.) against Fusarium oxysporum f. sp. cepa by target metabolite profiling. Plant Sci. 246, 128–138. https://doi.org/10.1016/j.plantsci.2016.02.008.

Akhter, A., Hage-Ahmed, K., Soja, G., Steinckelner, S., 2015. Compostand biochar alter mycorrhization, tomato root exudation, and development of Fusarium oxysporum Lsp. lycopersici. Front. Plant. Sci. 6, 529. https://doi.org/10.3389/fpls.2015.00529.
Kurabachew, H., Wydra, K., 2014. Induction of systemic resistance and defense-related enzymes after elicitation of resistance by rhizobacteria and silicon application against Ralstonia solanacearum in tomato (Solanum lycopersicum). Crop Prot. 57, 1–7. https://doi.org/10.1016/j.cropro.2013.10.021.
Landa, B.B., Cachinero-Diaz, J.M., Jimenez-Diaz, R.M., Alabouvette, C., 2002. Effect of fusaric acid and phytoanticipins on growth of rhizobacteria and Fusarium oxysporum. Cen. J. Microbiol. 48 (11), 971–985.
Lewandowski, T.J., Dunfield, K.E., Antunes, P.M., 2013. Isolate identity determines Landa, B.B., Cachinero-Diaz, J.M., Lemanceau, P., Jimenez-Diaz, R.M., Alabouvette, C., 2002. Effect of fusaric acid and phytoanticipins on growth of rhizobacteria and Fusarium oxysporum. Cen. J. Microbiol. 48 (11), 971–985.
Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplast. Plant Cell Physiol. 22, 867–880.
Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplast. Plant Cell Physiol. 22, 867–880.
Nelson, P.E., Toussoun, T.A., Marasas, W.F.O., 1983. Fusarium Species: An Illustrated Determinations of total carotenoids and chlorophyll a and b of leaf extracts in different solvents. Biochem. Soc. Trans. 11, 591–592.
Lima, L.K.S., de Jesus, O.N., Soares, T.L., de Oliveira, S.A.S., Haddad, F., Girardi, E.A., 2019. Water deficit increases the susceptibility of yellow passion fruit seedlings to fusarum wilt in controlled conditions. Scientia Horticulutae 243, 609–621.
Mcelrone, A.J., Sherald, J.L., Forseth, I.N., 2002. Interactive effects of water stress and xylem-limited bacterial infection on the water relations of a host vine. J. Exp. Biol. 54 (381), 419–430.
Mehboob, A.S.M., Matsubara, Y., 2012. Tolerance to Fusarium root rot and changes in antioxidative ability in mycorrhizal asparagus plants. Hort Sci. 47 (3), 356–360.
Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplast. Plant Cell Physiol. 22, 867–880.
Nelson, P.E., Toussoun, T.A., Marasas, W.F.O., 1983. Fusarium Species: An Illustrated Determinations of total carotenoids and chlorophyll a and b of leaf extracts in different solvents. Biochem. Soc. Trans. 11, 591–592.
Nirmaladevi, D., Sirnivas, C., 2012. Cultural, morphological and pathogenicity variations in Fusarium oxysporum f. sp. lycopersici causing wilt of tomato. Batman Univ. J. Life Sci. 2 (1), 1–16.
Nimaladevi, D., Sirinivas, C., 2012. Cultural, morphological and pathogenicity variations in Fusarium oxysporum f. sp. lycopersici causing wilt of tomato. Batman Univ. J. Life Sci. 2 (1), 1–16.
Petti, C., Reiber, K., Ali, S.S., Berney, M., Doohan, F.M., 2012. Auxin as a player in the biocostrol of Fusarium head blight disease of barley and its potential as a disease control agent. BMC Plant Biol. 12, 224.
Poso, M.J., Jung, S.C., López-Ráez, J.A., Azcon-Aguilar, C., 2010. Impact of arbuscular mycorrhizal symbiosis on plant response to biotic stress: the role of plant defence mechanisms. In: Koltai, Kapulnik, Y. (Eds.), Arbuscular Mycorrhizas: Physiology and Function, Amsterdam: Springer, pp. 193–207.
Qin, H.Z., Xing, H.C., Bin, Z.Z., Rong, Z.Z., Song, W.H., 2007. Changes of antioxidative enzymes and cell membrane osmosis in tomato colonized by arbuscular mycorrhizae under NaCl stress. Colloid. Surf. B Biointerf. 59, 128–133. https://doi.org/10.1016/j.colsurfb.2007.04.023.
Rasool, S., Ahmad, A., Siddiqi, T.O., Ahmad, P., 2013. Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. Acta Physiol. Plant. 35, 1039–1050. https://doi.org/10.1007/s11738-012-1142-4.
Ruiz-Lozano, J.M., 2003. Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. Mycorrhiza 13, 309–317. https://doi.org/10.1007/s00572-003-0237-6.
Sheng, M., Tang, M., Chen, H., Yang, B., Zhang, F., Huang, Y., 2008. Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. Mycorrhiza 18, 287–296. https://doi.org/10.1007/s00572-008-0180-7.
Smart, R.E., Bihgham, G.E., 1974. Rapid estimates of relative water content. Plant Physiol. 53, 258–260.
Smith, I.K., Vierheller, T.L., Thurne, C.A., 1988. Assay of glutathione reductase in crude tissue homogenates using 5,5’-dithiobis (2-nitrobenzoic acid). Anal. Biochem. 175, 408–413.
Song, Y., Chen, D., Lu, K., Sun, Z., Zeng, R., 2015. Enhanced tomato disease resistance primed by arbuscular mycorrhizal fungus. Front Plant Sci. 6, 786. https://doi.org/10.3389/fpls.2015.00786.
Stefan, E., 2005. Thin-layer chromatography-an appropriate method for fusaric acid estimation. Biologia Bratislava. 60 (1), 104.
Stutz, J.C., Morton, J.B., 1996. Successive pot cultures reveal high species richness of arbuscular endomycorrhizal fungi in arid ecosystems. Can. J. Botany 74, 1883–1889.
Summerrell, B., Salleh, B., Leslie, J., 2003. A utilitarian approach to Fusarium identification. Plant Dis. 87, 117–128.
Tang, M., Chen, H., Huang, J.C., Tao, Z.Q., 2009. AM fungi effects on the growth and physiology of Zea mays seedlings under diesel stress. Soil Biol. Biochem. 41, 936–940. https://doi.org/10.1016/j.soilbio.2008.11.007.
Thakker J.N., Patel S., Dhandhuka P.C., 2013. Induction of defense-related enzymes in banana plants: effect of live and dead pathogenic strain of Fusarium oxysporum f. sp. cubenae. ISRN Biotechnology. Article ID 601303, 6 pages. https://doi.org/10.5402/2013/601303.
Utobu, E.B., Ogbo, E.N., Nwogbaga, A.C., 2011. Techniques for extraction and quantification of arbuscular mycorrhizal fungi. Libyan Agric. Res. Center Int. 2, 68–78.
Valiyodan, B., Ye, H., Song, L., Murphy, M., Shannon, J.G., Nguyen, H.T., 2017. Genetic diversity and genomic strategies for improving drought and waterlogging tolerance in soybeans. J. Exp. Bot. 68, 1835–1849. https://doi.org/10.1093/jxb/erw433.
Vellosillo, T., Vicente, J., Kulašekarán, S., Hamberg, M., Castresana, C., 2010. Emerging complexity in reactive oxygen species production and signaling during the response of plants to pathogens. Plant Physiol. 154, 444–448.
Wu, H.S., Bao, W., Liu, D.Y., Ning, L., Ying, R.R., Raza, W., Shen, Q.R., 2008. Effect of fusaric acid on biomass and photosynthesis of watermelon seedlings leaves. Caryologia 61(3), 258–268.
Yadeta, K.A., Thomma, B.P.H.J., 2013. The xylem as battleground for plant hosts and vascular wilt pathogens. Front. Plant Sci. 4, 97. https://doi.org/10.3389/fpls.2013.00097.
Zhang, D., Song, H., Cheng, H., Hao, D., Wang, H., Kan, G., Jin, H., Yu, D., 2014. The acid phosphatase encoding gene GmACP1 contributes to soybean tolerance to low-phosphorus stress. PLoS Genet 10, e1004061. https://doi.org/10.1371/journal.pgen.1004061.