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ORIGINAL ARTICLE

Protection Against Fusarium Head Blight: Important Defense Mechanisms Studied in Three Regenerated Egyptian Wheat Cultivars

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Fusarium graminearum is one of the major global pathogens of cereals and is considered the main causal agent of *Fusarium* head blight (FHB) disease in wheat. *F. graminearum* leads to severe losses in grain yield and quality of wheat. In the present study, immature embryos of three Egyptian wheat cultivars (Giza 164, Sids 1 and Bani Suef 6) have been used to highlight the physiological changes in wheat plants in response to treatment with different concentrations of Fugal Culture Filtrate (5%, 10%, 20% and 40%). Samples were taken at 5, 10 and 15 days after inoculation. The three studied wheat cultivars exhibited different responses for inoculation with different concentrations of FCF. However, inoculation with 20% FCF significantly increased the activities of antioxidant enzymes (catalase, peroxidase and ascorbate peroxidase) in shoots of wheat cultivars compared with uninoculated ones. In addition, proline and secondary metabolites (total phenol and flavonoids) significantly increased throughout the experimental period. Our results also proved that in addition to the common protective mechanisms detected in all the infected cultivars there are cultivar – dependent physiological changes exhibited by wheat plant during abiotic stress.

Key words: Antioxidant enzymes, Fusarium graminearum, phenylalanine ammonia lyase, regenerated plant, secondary metabolites, wheat.

Abbreviations: APX: Ascorbate peroxidase, CAT: catalase, FCF: Fusarium culture filtrate, FHB: Fusarium head blight, LSD: Least significant difference, MS: Murashige and Skoog, PAL: Phenylalanine ammonia lyase, POX: Peroxidase, PR: Pathogenesis related proteins, PVP: Polyvinyl pyrrodine, ROS: Reactive oxygen species.

Wheat is one of the most important crops and is counted among the “big three” cereal crops (rice, wheat and maize). Wheat is produced on more than 18% of the arable land in the world (FAOSTAT data 2014). Wheat is one of the most abundant sources of energy and nourishment for mankind.

Wheat holds a number one position in human nutrition within the world. Therefore, sustained wheat production is necessary to make sure world food security. Wheat subjected lots of biotic and abiotic stresses; plant diseases are a major impediment to the production and quality of important foodstuffs. The International Maize and Wheat Improvement Center (CIMMYT) has identified *Fusarium* head blight (FHB) as the major factor limiting wheat production in many parts of the world. FHB has a great economic impact on the wheat industry due to the reduced grain yield and quality (Kazan et al., 2012)

The genus *Fusarium*, common soil saprophytes or plant pathogens, comprises a large number of species. They are common pathogens in small grains of wheat, barley, oats, triticale and rye and cause various disease such as: foot and root rot, crown rot, vascular wilt, seedling blight, cereal ear rot, fruit rot and *Fusarium* head blight (FHB) (Naz, 2007). FHB of small grains is caused primarily by *Fusarium graminearum* and *Fusarium culumolum* that are ascomycetes.

There are factors that affect FHB management such as selecting resistant wheat varieties, practicing cultivation, planting high-quality seed, and fungicide applications. Producers are encouraged to select varieties that have some level of FHB resistant.

When plants are exposed to pathogens, only small number of pathogens is able to provoke disease in a certain species (compatible response), whereas most pathogens aggressors are recognized and blocked in their penetration by plant-defenses (incompatible response) (De Gara, 2003). Plants produce various stress compounds by activating a wide variety of productive mechanisms designed to prevent pathogen replication and spread. The expression of antimicrobial proteins in plants that are not normally produced may have a greater potential to limit pathogen infection or growth. In compatible or incompatible interactions between plants and microorganisms, salicylic acid pathway, jasmonic acid pathway and shikimic acid pathway are involved in plant defense. Shikimic acid pathway is responsible for the secondary metabolites such as lignin, phenolic phytoalexins that adding mechanical rigidity and strength to cell walls and providing barriers to inhibit pathogens (Zheng *et al*., 2005).

The production of reactive oxygen species (ROS) is one of the earliest cellular responses following successful pathogen recognition. Generally when the level of antioxidants processes and detoxification mechanisms are lesser than the level of ROS accumulation, the cellular damage occurs through lipid peroxidation, membrane fatty acids and ruin macromolecules like pigments, proteins, nucleic acids and lipids (Torres *et al*., 2006). The rapid accumulation of plant ROS at the pathogen attack site, a phenomenon called oxidative burst, is toxic to pathogens directly and can lead to a hypersensitive response that results in a zone of host cell death which prevent further spread of pathogens. Plants have evolved

enzymatic and non-enzymatic defense mechanisms in order to reduce oxidative damages by detoxifying free radical (Borden and Higgin, 2002; EL-Khallal, 2007; Venkatesh *et al*., 2013).

Phenyl alanine ammonia lyase (PAL) is the most important enzyme in phenylpropanoid pathway catalyzing the transformation of L-phenylalanine into *trans*-cinnamic acid by deamination, which is the prime intermediary in the biosynthesis of phenolics, phytoalexin and lignin. A very rapid induction of PAL could be occurred during biotic and abiotic stresses. After pathogen infection, PAL activity increased leading to the formation of phenypropanoid phytoalexines (Boubakri *et al*., 2013).

Proline can serve as a protector of enzyme denaturation, as a stabilizer of protein synthesis machinery. Proline plays an important role in protecting the subcellular structures and mediating osmotic adjustment in plant under stressed conditions (Bagdi and Shaw, 2013). Accumulation of the amino acid proline is one of the indications to plant adaptation to unfavorable conditions. It regulates the osmotic equilibrium between the cytoplasm and vacuole. Proline can now be added to an elite list of non-enzymatic antioxidants that microbes, animals, and plants need to counteract the inhibitory effects of ROS so that proline acting as s singlet oxygen quencher (Abdel-fattah and Asrar, 2012). Moreover, proline has been shown to function as a molecular chaperone able to protect protein integrity and enhance the activities of different enzymes (Rajendrakumar *et al*., 1994).

However, our understanding of these defensive mechanisms is still limited. The present work aimed to identify the induced defense mechanisms in wheat plants in response to inoculation with *Fusarium graminearum*. Induced resistance could be exploited as an important tool for the pathogen control. The elicitors of induced responses can be sprayed on crop plants to build up the natural defense system against damage caused by that pathogen. The induced responses can also be engineered genetically, so that the defensive compounds are constitutively produced in plants those are challenged by that pathogen.

MATERIALS AND METHODS

Plant materials. Seeds of two bread wheat (*Triticium aestivium* L.) cultivars; Giza 164, Sids 1 and one durum wheat (*Triticium durum* L.) cultivar; Bani Suef 6 were kindly provided by Field Crop Research Institute (FCRI), Agricultural Research Center (ARC), Giza, Egypt.

Fusarium graminearum was obtained from Mycological Center, Faculty of Science, Assiut University, Egypt.

Growth conditions. This experiment was carried out in the Plant Cellular and Molecular Genetics Laboratory (PCMG), Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza, Egypt. Immature caryopsis of the three Egyptian wheat cultivars Giza 164, Sids 1 and Bani Suef 6 were collected approximately two weeks post anthesis. Immature grains for each cultivar were surface sterilized (by soaking for 1 min in 70% (v/v) ethanol followed by 20% commercial Clorox (5.25% Sodium hypochlorite) supplemented with few drops of Tween 20, and washed five times with sterile ddH_2O). Immature embryos were aseptically isolated and cultured with the scutellum side up onto the callus induction medium. Consequently, immature embryos were cultured

in the dark at 25°C on callus induction medium for two weeks then subculture for another two weeks. Four week-old calli of different cultivars were transferred onto plant regeneration medium at 24°C under 16 h / 8 h light/ dark cycle for a month with subculture of two weeks interval.

The regenerated plantlets of each wheat cultivar were transferred into treated-rooting medium inoculated with *F. graminearum* extract at different concentrations (5, 10, 20 and 40% v/v) and kept at the same condition of regeneration phase. Physiological parameters were determined including: total soluble proteins; the activity of antioxidant enzymes (catalase; CAT, Ascorbate peroxidase; APX and peroxidase; POX) ; PAL activity, proline content; phenols content and flavonoids content at 5, 10 and 15 days after inoculation. Statistical analysis of physiological parameters (5 replicates each) was analyzed using Duncan test at 5% of probabilities according to SPSS program (version 17).

Wheat Regeneration

Isolation and culturing of the explant. Immature embryos for each cultivar were aseptically isolated with forceps under sterile conditions in the laminar air - flow hood. Fifty immature embryos were cultured with the scutellum side up onto the callus induction medium modified for wheat cell culture (Weeks et al. 1993; Fahmy et al. 2006). Basically, it contains MS (Murashige and Skoog, 1962) medium salt, supplemented with 2 mg/L 2,4-D as a source of auxin, 0.15 g of L-Asparagine, 0.1g of myo-inositol, 50 mg thiamine-HCL, 20 g sucrose and adjusted to 5.8 pH and solidified by 2.5 g/L phytagel. Immature embryos were cultured in the dark at 25°C on the callus induction medium for two weeks for infection.

Regeneration Two week-old calli were transferred onto plant regeneration medium in Petri dishes containing 50 ml of MS medium (MS salts with vitamins, 30 g / L sucrose and 3 g / L phytagel) supplemented with 1.5 mg / L TDZ at 24°C and kept under 16 h / 8 h light / dark cycle. Calli of different cultivars were subcultured twice times with two weeks interval, then the regenerated plantlets of each calli were transferred onto rooting medium which was half strength MS medium (MS salts with vitamins, 30 g / L: sucrose and 2.5 g / L phytagel), and kept in the growth chamber under the same light and growth conditions.

Fungus maintenance medium. The *Fusarium graminearum* was cultured and maintained on Czapek's medium and incubated at 28 ± 1˚C in the dark. The Czapek's medium contained for each liter: 2 g $NaN0_3$; 1 g K_2HPO_4; 0.5 g $MgSO_4.7H_2O$; 0.5 g KCl; 0.01 g Fe-EDTA; 5 g yeast extract; 30 g sucrose and solidified with 15 g agar (Jiao et al. 2008).

Culture of fungus inoculum. The fungus was sub-cultured on Petri-dishes containing sterilized, solidified Czapek's medium and kept for 7 days in the dark growth chamber at 28 ± 1°C. Sterile inoculation loop were used to transfer a *F. graminearum* colonies from the Petri-dish into sterile flasks, each with 250 ml of liquid Czapek's medium without antibiotics and phytohormones. All flasks (12 flasks) were incubated in dark for two weeks at 28 ± 1°C in shaking orbital incubator at 100 rpm to provide aeration.

Fungus extract. At the end of the incubation period, the culture was filtered through Whatman number one filter paper to remove conidia and mycelia. The pH of filtrate was adjusted to 5.8 and then passed through

micro-Millipore filter to sterilize the filtrate, yielding of *F. graminearum* extract which was used as stock for the inoculation.

Inoculation and incubation. Rooting medium was prepared by replacing part of ddH_2O with equivalent volume of culture filtrate. The pH of the medium was adjusted to 5.8 before autoclaving, cooled (60 ± 4°C) then supplemented with different concentrations of *F. graminearum* extract (5.0, 10.0, 20.0 and 40.0 % v/v), under aseptic conditions. Four weeks-old regenerated plants from each cultivar were transferred into treated-rooting medium (with *F. graminearum* culture filtrate) or into non-treated rooting medium (without *F. graminearum* culture filtrate) as control and kept at the same condition of regeneration phase. Effect of fungal filtrate was studied by collecting the samples at 5, 10 and 15 days after inoculation.

Protein determination. Total soluble proteins were extracted from the samples. The method described by Guy et al. (1992), the shoot tissue was ground in liquid nitrogen and extracted with buffer containing ice-cold 50 mM Tris-HCl, pH 7.5; 2 mM EDTA and a 0.04% (v/v) 2-β-mercaptoethanol. The homogenate was centrifuged at 14000 rpm for 20 min at 4°C. Total protein amount was determined using the dye-binding assay of Bradford (1976) with bovine serum albumin as a standard.

Determination of antioxidant enzymes activities

Extraction of enzymes. Samples (0.1 g of shoots) were ground and homogenized in 4 ml ice-cold extraction buffer (100 mM KH_2PO_4 / K_2HPO_4 (pH 7.8), 60 mg polyvinyl-pyrrolidone (PVP) and 5 mM ascorbate. The homogenate was centrifuged at 12,000 x g for 15 min at 4°C. The supernatant was used for the determination of peroxidase (POX), ascorbate peroxidase (APX) and catalase (CAT) activities.

Peroxidase. POX (EC 1.11.1.7) activity was assayed by the method of Velikova *et al.* (2000). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.2% of guaiacol (w/v) and 0.04 ml of enzymes extract. The reaction mixture was incubated at room temperature for 5 min after the addition of 3mM H_2O_2. The absorbance was then measured at 470 nm. The activity of POX was calculated from the rate of formation of guaiacol dehydrogenation product (GDHP) using the extinction coefficient of 26.6 mm^{-1} cm^{-1}, and the activity was expressed as µM GDHP / min / g fresh weight.

Catalase. CAT (EC 1.11.1.6) activity was measured according to Aebi (1984). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.1 ml enzyme extract and 0.035% H_2O_2. The activity of CAT was calculated based on the decomposition of H_2O_2 measured as a decline in the absorbance at 240 nm. The activity was calculated using the extinction coefficient of 40.84 mm^{-1} cm^{-1}, and expressed as µM H_2O_2 reduced / min / g fresh weight.

Ascorbate peroxidase. For APX (EC 1.11.1.11) assay, the reaction mixture contained 0.5 mM ascorbic acid, 0.1 mM EDTA and 0.1 mL of enzyme extract. The reaction was initiated when adding 1.5 mM H_2O_2. The absorbance of the reaction mixture was measured at 290 nm 2 min after H_2O_2 was added. The activity of APX was calculated using the extinction coefficient of 3.98 $mm^{-1}cm^{-1}$and the activity was expressed as µM ascorbate oxidized / min / g fresh weight (Asada, 1992).

Assay of Phenylalanine Ammonia Lyase. To

determine Phenylalanine ammonia lyase (PAL) activity (EC 4.3.1.5); 0.15 g of shoot was homogenized in 1.5 ml of ice-cold 0.1M sodium borate buffer, pH 7.0 containing 1.4 mM of 2-mercaptoethanol and 0.1g of insoluble PVP. The homogenate was centrifuged at 12,000 × g for 30 min and the supernatant was collected for enzyme assay. PAL activity was determined based on the rate of conversion of L-phenylalanine to *trans*-cinnamic acid at 290 nm (Dickerson *et al*., 1984). Sample containing 0.4 ml of enzyme extract was incubated with 0.5ml of 0.1M borate buffer, pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 minutes at 30°C. The activity of PAL was expressed as µM *trans*-cinnamic acid synthesized / min / g fresh weight.

Measurement of free proline. Proline content in shoots and roots of the three wheat cultivars was determined according to the method of Bates et al. (1973). Fresh weight of tissue was ground with 10 ml of 3% sulphosalicylic acid then the homogenate was filtered through two sheets of 3 mm Whatman paper. Two ml of the filtrate was mixed with 2 ml of ninhydrin reagent (1.25 g ninhydrine in 3 ml glacial acetic acid and 20 ml 6 M phosphoric acid with agitation, until dissolved) and 2 ml of glacial acetic acid. The reaction mixture was incubated in water bath for 1 h at 100°C then tube was immediately transferred to ice bath. Four ml of the toluene was added to the mixture, mixed vigorously with a test tube stirrer for 15-20 sec. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and absorbance read at 520 nm using toluene as a blank. The results were expressed as µg /g fresh weight.

Determination of phenol content. Shoot tissue (0.1 g) was extracted with chilled 1.5 ml 80% methanol and 1% HCl at room temperature with shaking for 2 h. The extract was centrifuged at 3000 g for 10 min. Supernatant was used to determination phenol content.

Free phenol content in the extract of three wheat cultivars were immediately determined using the Folin-Ciocalteu's method by Hung *et al.* (2009), with a modification. To the 150 µL of the extract 750 µL of distilled water were added. This solution was oxidized with 300 µL of Folin-Ciocalteu's reagent and then neutralized with 300 µL of 20 % sodium carbonate solution (20 % w/v). Thoroughly mixed samples were allowed to stand for 120 min at dark place at ambient temperature. The solution was centrifuged for 10 min at 12000 rpm and the absorbance of the clear supernatants was measured at 765 nm using a spectrophotometer (Spectro. DioRAD Smartspec™ 3000). The content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA / g of extract).

Determination of Flavonoids. Flavonoids were extracted according to the method reported by (Abeysinghe *et al.,* 2007). Shoot tissue (0.1 g) was ground with liquid nitrogen, 1 ml of methanol-water (80:20, v/v) was added, then covered with aluminium foils and finally placed into water bath for extraction at 80°C for 1 h. The solution was filtered through Whatman filter paper and filtrate was evaporated in an oven at 60 ° C. The residue was dissolved in the 1 ml of methanol (80%).

The content of flavonoids in the examined plant extracts was determined using spectrophotometric method (Quettier *et al*., 2000). The sample contained 1 ml of methanol solution of the extract in the concentration of 1 mg / ml and 1 ml of 2% $AlCl_3$ solution dissolved in

methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at 415 nm. The content of flavonoids in extracts was expressed in terms of rutin equivalent (mg of RU / g of extract).

RESULTS

Total soluble proteins. Table (1) showed that inoculation wheat plants cv. Giza 164 with *F. graminearum* extract (5, 10, 20 and 40% v/v) caused, in general, a highly significant reduction in total soluble protein during the experimental period. The low concentration of fungal culture filtrate (10%) recorded the highest reduction at the three ages. The highest reduction was 41.14% at five days from inoculation. But the 20% of fungal culture filtrate showed different behavior, the total soluble protein was slightly increased at the second and third ages of experiment reaching 6.83 % and 19.23% over the control plants, respectively.

On the other hand, inoculation with *F. graminearum* extract at different concentrations caused significantly increases in total soluble protein in the shoots of wheat plants cv. Sids1. The highest amount of soluble protein was observed at 20% of fungal culture filtrate at the third age recording 74.36% over the control plants.

In the case of *Triticium durum* L. cv. Bani Suef 6, total soluble protein increased gradually in the shoots of wheat cultivar that inoculated with different concentrations of *F. graminearum* extract. The highest increment was at 20% of *F. graminearum* extract at 5, 10 and 15 days after inoculation reaching 66.75 %, 82.02 % and 146.26 % over those of the uninoculated plants, respectively. At 40% of *F. graminearum* extract, shoots of wheat cultivar showed increment in the soluble protein but this increment was markedly lower compared with 20% of *F. graminearum* extract at the three ages.

Antioxidant enzymes

Catalase. Catalase activity was significantly increased in shoots of the three studied wheat cultivars Giza 164, Sids 1 and Beni suef 6 in response to inoculation. The maximum catalase activity was recorded in most cases at 20% of *F. graminearum* extract (Figure 1). The increase was evaluated by 49.63% and 102.48% in Giza 164 after 10 and 15 days of inoculation, respectively, 132.86% in Sids 1 after 15 days of inoculation and 50.26%, 77.23% and 112.02% at 5, 10 and 15 days from inoculation, respectively in cultivar Bani Suef 6.

Meanwhile, 40% FCF caused the maximum increase in catalase activity (89.64% and 38.12%) in cultivar Sids 1 after 5 and 10 days of inoculation and in Giza 164 after 5 days of inoculation (58.52% over the control value.

Peroxidase. Data in Figure (2) showed changes in the guaiacol peroxidase activity in uninoculated wheat plants of the three cultivars during the experimental period.

The inoculation with *F. graminearum* extract induced significant increase in the activity of guaiacol peroxidase in shoots of wheat cultivar Giza 164. The maximum value of guaiacol peroxidase activity was observed at 40% in the first age (12.19%), at 20% in the second and third ages (20.24% and 42.98%, respectively) as compared with untreated plants. However, shoots of wheat cultivar Giza 164 inoculated with 5% of *F. graminearum* extract exhibited decreases in the guaiacol peroxidase activity at first and second

ages evaluated by 6.49% and 13.65%, respectively below the control value.

In case of *Triticium aestivium* L. cv. Sids 1, the shoots that inoculated with different concentrations of *F. graminearum* extract showed general increases in the activity of guaiacol peroxidase. The high activity of guaiacol peroxidase was shown at 40% of *F. graminearum* extract at the 5 and 10 days after inoculation reaching 76.22% and 85.49%, over the control, respectively. However, in the third age, the high activity of guaiacol peroxidase recorded at 20% of *F. graminearum* extract 97.06% increase over that of the uninoculated plants.

In contrast, *Triticium durum* L. cv.Bani Suef 6, shoots that inoculated with different concentration of *F. graminearum* extract (5%, 10%, 20% and 40%) showed reduction in the activity of guaiacol peroxidase at the first age followed by increases at the second and third ages.

Ascorbate peroxidase. From results presented in Figure (3) it is obvious that treatment with different concentrations of *F. graminearum* extract caused significant marked increases in the activity of ascorbate peroxidase in the shoots of wheat cultivar Giza 164 reaching the maximum level (247.19%) at 20% FCF at the third age.

The inoculation with different concentration of *F. graminearum* extract stimulated ascorbate peroxidase activity in the shoot of wheat cultivar Sids 1 as compared with uninoculated plants. Data in Figure (3) showed that the activity of ascorbate peroxidase gradually increased in the shoot inoculated with *F. graminearum* extract at 5%, 10% and 20% and decreased in response to treatment with 40% of the fungal extract at 5, 10 and 15 days after inoculation.

In case of Beni Suef 6 cultivar, the results showed that the inoculation with *F. graminearum* extract at different concentrations caused a gradual increase in the ascorbate peroxidase activity in the inoculated shoots along the experimental time. The highest value of enzyme activity observed at 20% of *F. graminearum* extract recording 26.31%, 101.96% and 144.23% at the first, second and third age, respectively above that of the control plants.

Phenylalanine ammonia lyase. Figure (4) showed that there was a significant increase in the PAL activity in the uninoculated shoots of the three wheat cultivars at the three ages (5, 10 and 15 days). The inoculation with *F. graminearum* extract significantly increased the activity of PAL in the shoots of wheat Giza 164 cultivar from the 5^{th} day up to 15^{th} day after inoculation. The concentration (10%) of *F. graminearum* extract exhibited a high enhancement (55.16%) in the PAL activity at the first age, while higher increases were observed in the PAL activity at the second (63.32%) and third ages (134.89%) using 20% of *F. graminearum* extract.

In the case of inoculated shoots of wheat cultivar Sids 1, the maximum PAL activity was detected in plant infected with 10% FCF at 5 days after inoculation, 40% FCF at 10 DAI and 20% at 15 DAI and was estimated by 83.62%, 89.89% and 227%, respectively over the control value.

In contrast inoculation of *Triticium durum* L. cv. Bani Suef 6, with 5, 10 and 40 % FCF caused, in most cases,

a significant reduction in PAL activity. Whereas, 20% of *F. graminearum* extract caused a significant increase in PAL activity by 11.21%, 8.32% and 29.95% at 5th, 10th and 15th days, respectively as compared with the control plants.

Proline content. The data presented in Table (2) showed that proline content significantly increased in inoculated shoots of the three cultivars of wheat plants throughout the experimental period. At 5 DAI the maximum proline content was detected in wheat shoots treated with 40% FCF and was calculated by 245% in Giza 164, 203% in Sids 1 and 227% in Bani Suef 6 compared to the corresponding control value. Whereas, at the end of the experimental period (15 DAI) the highest proline content was obtained in response to treatment with 20% FCF and was estimated by 349%, 313% and 217% compared to corresponding control in cultivars Giza 164, Sids 1 and Bani Suef 6, respectively.

Phenol content. It is clear from Table (3) there was slight change in the phenol content in uninoculated shoots of three wheat cultivars. Interesting, At 5th day after inoculation in bread wheat cultivars, Giza 164 and Sids 1 the phenol content decreased showing the highest reduction in the phenol content at 40% of *F. graminearum* extract (29.71% in Giza 164 and 22.35% in Sids1). At 10th day in the bread wheat, the phenol content significantly increased in inoculated shoots and reached its maximum level (49.70%) at 20% of *F. graminearum* extract in case of cultivar Giza 164, in this meantime, the highest level of phenol in case of cultivar Sids 1 was 26.76% at 40% of *F. graminearum* extract as compared with uninoculated plants. At 15th day after inoculation with different concentrations of *F. graminearum* extract in Giza 164 and Sids 1 cultivars, the maximum level of phenol content was shown at 20% of *F. graminearum* extract in both cultivars reaching 189.43% in Giza 164 and 123.22% in Sids 1.

In case of *Triticium durum* L. cv. Bani Suef 6, inoculated shoots showed different behavior at 5th day after inoculation with *F. graminearum* extract. The highest level of phenol was 9.38% at 10% of *F. graminearum* extract and the reduction of phenol content exhibited 3.13% at 40% of *F. graminearum* extract as compared with control plants. At 10th day and 15th day, the inoculated shoots exhibited slight increase in the phenol content at 20% of *F. graminearum* extract with a maximum of 22.86% and 40.04%, respectively over the control level.

Flavonoid content. The data presented in Table (4) showed that in case of *Triticum aestivum* L. cv. Giza164 and Sids 1, inoculated shoots of both cultivars exhibited reduction in the flavonoid content with different concentration of *F. graminearum* extract at 5th day after inoculation reaching 43.64% at 20% of *F. graminearum* extract in Giza 164 and 29.45% at 10% of *F. graminearum* extract in Sids 1 as compared with control plants. At 10th and 15th days after inoculation, the flavonoid content increased in the shoots of both wheat cultivars. The highest level of flavonoid content in both wheat cultivars was shown with 20% of *F. graminearum* extract recording 36.94% at 10th day & 75.84% at 15th day after inoculation for cv. Giza 164, and 22.41% at 10th day and 127.93% at 15th day after inoculation in cv. Sids 1 as compared with uninoculated plants.

In case of *Triticium durum* L. cv.Bani Suef 6, the

flavonoid content was markedly increased in inoculated shoots plants with *F. graminearum* extract during the experimental period. The maximum value of flavonoid (90.45%) was observed at 40% of *F. graminearum* extract in 5th day after inoculation as compared with control plants. Interestingly, the highest flavonoid content was observed at 20% of *F. graminearum* extract recording 115.94% and 165.56%, respectively at 10th and 15th day after inoculation.

Table 1. Effect of different concentrations of *F. graminearum* culture filtrate on the total soluble protein of shoots of wheat (*Triticium aestivium* L.) cv. Giza 164, cv.Sids 1 and (*Triticium durum* L.) cv. Bani Suef 6 at 5, 10 and 15 days after inoculation. Values listed are expressed as mg / g fresh weight.

Cultivar	Treatment	5 DAI	% of control	10 DAI	% of control	15 DAI	% of control
Giza 164	Control	5.022 ± 0.076 a	100	5.241 ± 0.047 b	100	5.354 ± 0.084 b	100
	FCF 5%	3.954 ± 0.196 c	78.73	4.344 ± 0.039 d	82.88	4.821 ± 0.067 d	90.04
	FCF 10%	2.956 ± 0.112 d	58.86	3.339 ± 0.084 e	63.70	4.080 ± 0.089 e	76.20
	FCF 20%	4.570 ± 0.108 b	90.99	5.599 ± 0.196 a	106.83	6.384 ± 0.104 a	119.23
	FCF 40%	3.898 ± 0.154 c	77.61	4.582 ± 0.113 c	87.42	5.165 ± 0.076 c	96.46
	LSD 5%	0.175		0.139		0.106	
Sids 1	Control	2.304 ± 0.057 d	100	2.178 ± 0.132 c	100	2.329 ± 0.039 c	100
	FCF 5%	3.245 ± 0.113 b	140.84	3.308 ± 0.164 a	151.88	3.452 ± 0.089 b	148.21
	FCF 10%	3.446 ± 0.067 a	149.56	3.321 ± 0.174 a	152.47	3.980 ± 0.047 a	170.88
	FCF 20%	2.913 ± 0.054 c	126.43	2.975 ± 0.094 b	136.59	4.061 ± 0.057 a	174.36
	FCF 40%	3.270 ± 0.060 b	141.92	3.333 ± 0.049 a	153.03	3.547 ± 0.039 b	152.29
	LSD 5%	0.089		0.165		0.069	
Bani Suef 6	Control	1.167 ± 0.037 e	100	1.224 ± 0.018 e	100	1.193 ± 0.086 d	100
	FCF 5%	1.450 ± 0.049 d	124.25	1.556 ± 0.021 d	127.12	2.090 ± 0.049 c	175.18
	FCF 10%	1.782 ± 0.047 b	152.69	1.921 ± 0.037 b	156.94	2.693 ± 0.117 b	225.73
	FCF 20%	1.946 ± 0.060 a	166.75	2.228 ± 0.029 a	182.02	2.938 ± 0.086 a	246.26
	FCF 40%	1.638 ± 0.049 c	140.35	1.852 ± 0.028 c	151.30	1.933 ± 0.092 c	162.02
	LSD 5%	0.056		0.040		0.113	

FCF: Fungal culture filtrate DAI: Days after inoculation
Values are mean of three samples ± SD.
Different letters indicate significant variation between vertical readings for each cultivar at P = 0.05 according to Duncan's multiple range test

Table 2. Effect of different concentrations of *F. graminearum* culture filtrate on proline contentof shoots of wheat (*Triticium aestivium* L.) cv. Giza 164, cv.Sids 1 and (*Triticium durum* L.) cv.Bani Suef 6 at 5, 10 and 15 days after inoculation. Values listed are expressed as µg / g fresh weight.

Cultivar	Treatment	5 DAI	% of control	10 DAI	% of control	15 DAI	% of control
Giza 164	Control	0.946±0.039 e	100	1.038±0.035 e	100	1.165±0.040 d	100
	FCF 5%	1.142±0.034 d	120.71	1.534±0.053 d	147.78	0.969±0.035 e	83.17
	FCF 10%	1.661±0.067 c	175.58	2.099±0.072 c	202.21	3.229± 0.072 b	277.16
	FCF 20%	2.134±0.053 b	225.58	2.918±0.087 a	281.11	4.071± 0.106 a	349.44
	FCF 40%	2.318±0.069 a	245.03	2.480±0.052 b	238.92	2.814± 0.105 c	241.54
	LSD 5%	0.069		0.080		0.098	
Sids 1	Control	1.545±0.052 e	100	1.543±0.055 e	100	1.684±0.072 e	100
	FCF 5%	2.318±0.034 c	150.03	2.313±0.036 c	149.90	3.183±0.103 d	189.01
	FCF 10%	1.995±0.052 d	129.12	1.985±0.069 d	128.64	3.806±0.069 b	226.00
	FCF 20%	2.768±0.069 b	179.15	2.738±0. 117 b	177.44	5.271±0.053 a	313.00
	FCF 40%	3.137±0.072 a	203.04	3.143±0.083 a	203.43	3.425±0.060 c	203.38
	LSD 5%	0.069		0.098		0.089	
Bani Suef 6	Control	2.468±0.053 d	100	2.676±0.040 e	100	2.837±0.103 e	100
	FCF 5%	3.898±0.122 c	157.94	3.402±0.053 d	127.13	4.175±0.121 d	147.16
	FCF 10%	4.763±0.106 b	192.99	5.386±0.072 b	201.27	6.447±0.052 b	227.24
	FCF 20%	3.783±0.144 c	153.28	6.032±0.052 a	225.41	7.704±0.087 a	271.55
	FCF 40%	5.617±0.105 a	227.59	4.959±0.087 c	185.31	5.790±0.072 c	204.08
	LSD 5%	0.139		0.080		0.113	

Values are mean of three samples ± SD. Different letters indicate significant variation between vertical readings for each cultivar at P = 0.05 according to Duncan's multiple range test.

FCF: Fungal culture filtrate, DAI: Days after inoculation.

Table 3. Effect of different concentrations of *F. graminearum* culture filtrate on phenol contentof shoots of wheat (*Triticium aestivium* L.) cv. Giza 164, cv. Sids 1 and (*Triticium durum* L.) cv. Bani Suef 6 at 5, 10 and 15 days after inoculation. Values listed are expressed as mg / g fresh weight.

Cultivar	Treatment	5 DAI	% of control	10 DAI	% of control	15 DAI	% of control
Giza 164	Control	0.771 ±0.014 b	100	0.845 ±0.004 e	100	0.852 ±0.002 e	100
	FCF 5%	0.833 ±0.013 a	108.04	1.140 ±0.003 c	134.91	1.471 ± 0.004 d	172.65
	FCF 10%	0.632 ±0.004 d	81.97	1.011 ±0.001 d	119.64	1.847 ±0.003 b	216.78
	FCF 20%	0.653 ±0.006 c	84.69	1.265 ± 0.002 a	149.70	2.466 ±0.002 a	289.43
	FCF 40%	0.542 ±0.011 e	70.29	1.193 ± 0.004 b	141.18	1.560 ±0.007 c	183.09
	LSD 5%	0.010		0.003		0.004	
Sids 1	Control	0.537 ±0.003 a	100	0.553 ±0.002 c	100	0.564 ±0.004 e	100
	FCF 5%	0.496 ±0.004 b	92.36	0.525 ±0.003 d	94.93	0.661 ±0.003 d	117.19
	FCF 10%	0.430 ±0.001 d	80.07	0.475 ±0.004 e	85.89	0.934 ±0.003 c	165.60
	FCF 20%	0.446 ±0.007 c	83.05	0.657 ±0.005 b	118.80	1.259 ±0.004 a	223.22
	FCF 40%	0.417 ±0.010 e	77.65	0.701 ±0.004 a	126.76	0.987 ±0.003 b	175.00
	LSD 5%	0.005		0.004		0.003	
Bani Suef 6	Control	0.767 ±0.011 b	100	0.783 ±0.003 d	100	0.804 ±0.003 e	100
	FCF 5%	0.775 ±0.002 b	101.04	0.832 ±0.002 c	106.25	0.883 ±0.004 c	109.82
	FCF 10%	0.839 ±0.004 a	109.38	0.865 ±0.002 b	110.47	0.932 ±0.005 b	115.92
	FCF 20%	0.750 ±0.003 c	97.78	0.962 ±0.004 a	122.86	1.126 ±0.004 a	140.04
	FCF 40%	0.743 ±0.004 c	96.87	0.772 ±0.006 e	98.59	0.823 ±0.003 d	102.36
	LSD 5%	0.006		0.003		0.004	

FCF: Fungal culture filtrate DAI: Days after inoculation. Values are mean of three samples ± SD. Different letters indicate significant variation between vertical readings for each cultivar at P = 0.05 according to Duncan's multiple range test.

Table 4. Effect of different concentrations of *F. graminearum* culture filtrate on flavonoid content of shoots of wheat (*Triticium aestivium* L.) cv. Giza 164, cv.Sids 1 and (*Triticium durum* L.) cv. Bani Suef 6 at 5, 10 and 15 days after inoculation. Values listed are expressed as mg / g fresh weight.

Cultivar	Treatment	5 DAI	% of control	10 DAI	% of control	15 DAI	% of control
Giza 164	Control	0.220±0.004 a	100	0.268±0.004 e	100	0.278 ±0.003 e	100
	FCF 5%	0.131±0.002 c	59.54	0.308±0.004 c	114.92	0.356 ±0.004 d	128.05
	FCF 10%	0.132±0.005 c	60.00	0.321 ±0.003 b	119.77	0.426 ±0.003 b	153.23
	FCF 20%	0.124 ±0.001 d	56.36	0.367 ±0.006 a	136.94	0.489±0.006 a	175.89
	FCF 40%	0.208±0.004 b	94.54	0.292 ±0.002 d	108.95	0.375±0.003 c	134.89
	LSD 5%	0.003		0.004		0.004	
Sids 1	Control	0.163 ±0.003 b	100	0.174±0.001 d	100	0.179±0.002 d	100
	FCF 5%	0.159 ±0.004 c	97.54	0.184 ±0.000 c	105.74	0.159 ±0.003 e	88.82
	FCF 10%	0.115 ±0.002 e	70.55	0.192±0.001 b	110.34	0.318 ±0.003 c	177.65
	FCF 20%	0.176 ±0.004 a	107.97	0.213 ±0.001 a	122.41	0.408 ±0.004 a	227.93
	FCF 40%	0.148 ±0.002 d	90.79	0.171 ±0.00 1e	98.27	0.348 ±0.002 b	194.41
	LSD 5%	0.004		0.001		0.003	
Bani Suef 6	Control	0.199 ±0.002 e	100	0.207±0.004 e	100	0.212±0.003 e	100
	FCF 5%	0.274 ±0.001 d	137.68	0.317±0.004 c	153.14	0.418±0.003 c	197.16
	FCF 10%	0.308 ±0.004 c	154.77	0.354±0.002 b	171.01	0.497±0.007 b	234.43
	FCF 20%	0.335 ±0.010 b	168.34	0.447±0.004 a	21594	0.563±0.005 a	265.56
	FCF 40%	0.379 ±0.002 a	190.45	0.231±0.003 d	111.59	0.338±0.008 d	159.43
	LSD 5%	0.004		0.003		0.005	

FCF: Fungal culture filtrate DAI: Days after inoculation. Values are mean of three samples ± SD. Different letters indicate significant variation between vertical readings for each cultivar at P = 0.05 according to Duncan's multiple range test.

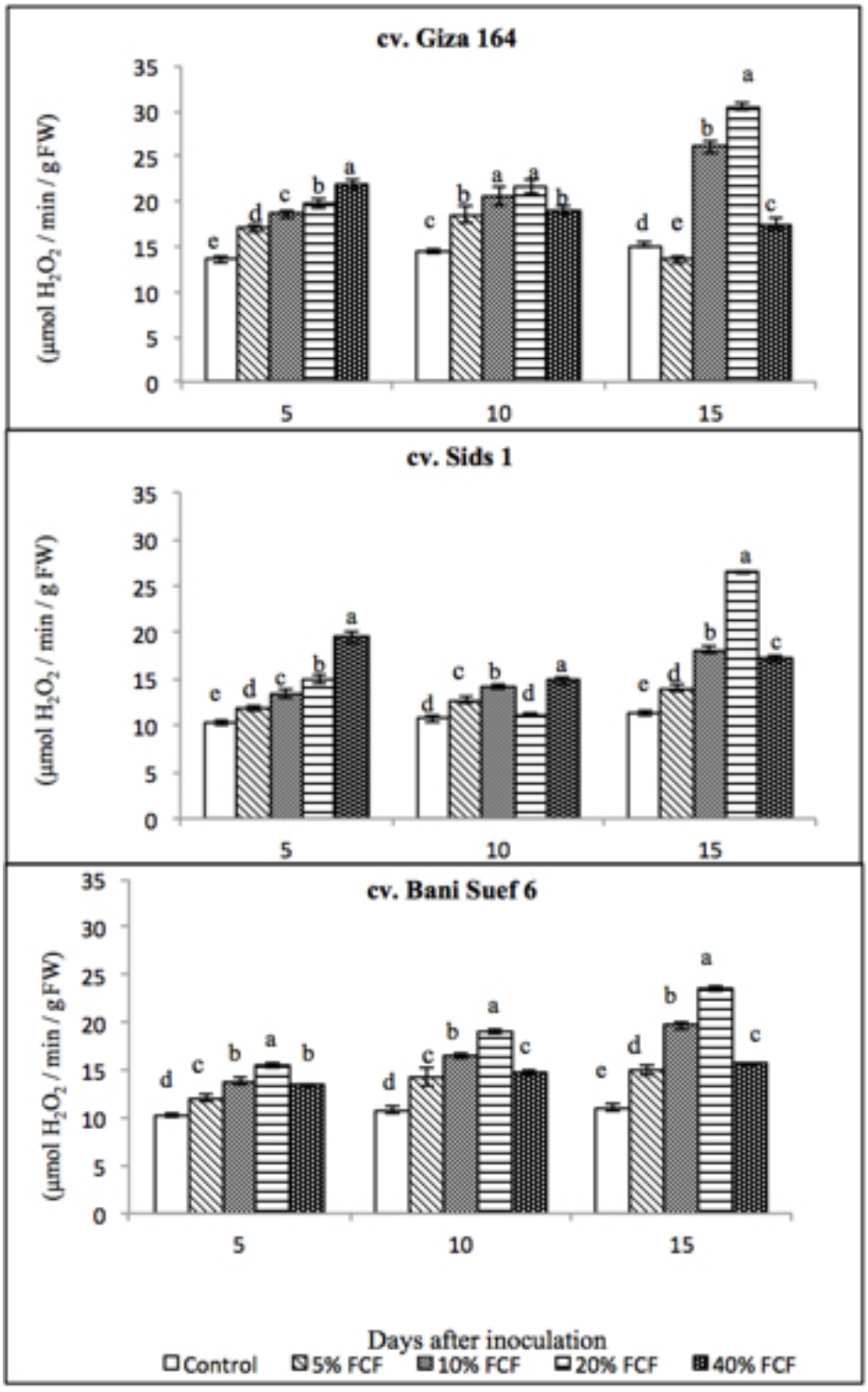

Figure 1. Effect of different concentrations of *F. graminearum* culture filtrate on catalase activity of shoots of wheat (*Triticium aestivium* L.) cv. Giza 164, cv. Sids 1 and (*Triticium durum* L.) cv. Bani Suef 6 at 5, 10 and 15 days after inoculation. Data are means of three samples ± SD. Within different time, different letters indicate significant variation according to Duncan's multiple range test at P = 0.05. FCF: Fungal cultures filtrate.

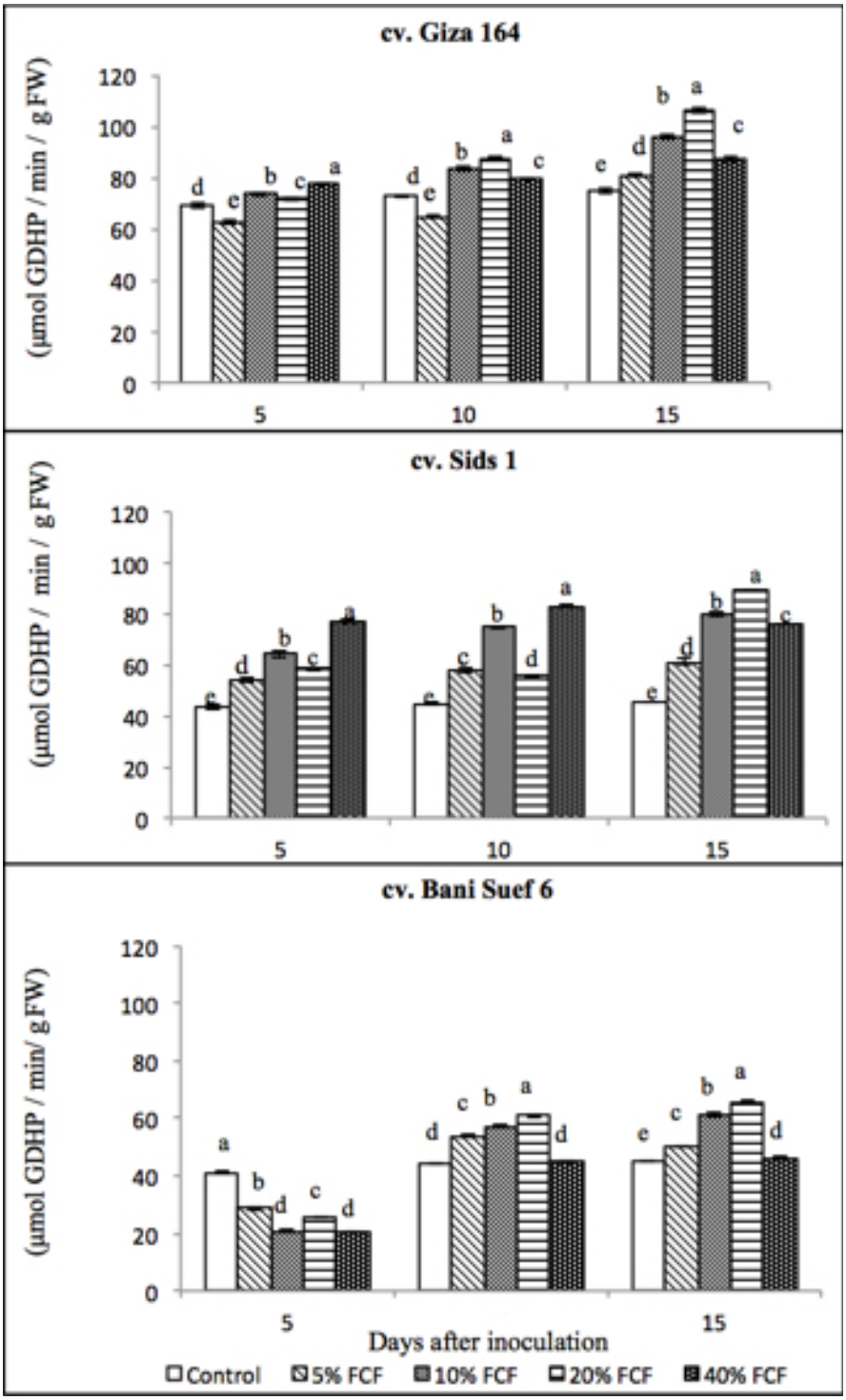

Figure 2. Effect of different concentrations of *F. graminearum* culture filtrate on peroxidase activity of shoots of wheat (*Triticium aestivium* L.) cv. Giza 164, cv. Sids 1 and (*Triticium durum* L.) cv. Bani Suef 6 at 5, 10 and 15 days after inoculation. Data are means of three samples ± SD. Within different time, different letters indicate significant variation according to Duncan's multiple range test at P = 0.05. FCF: Fungal cultures filtrate.

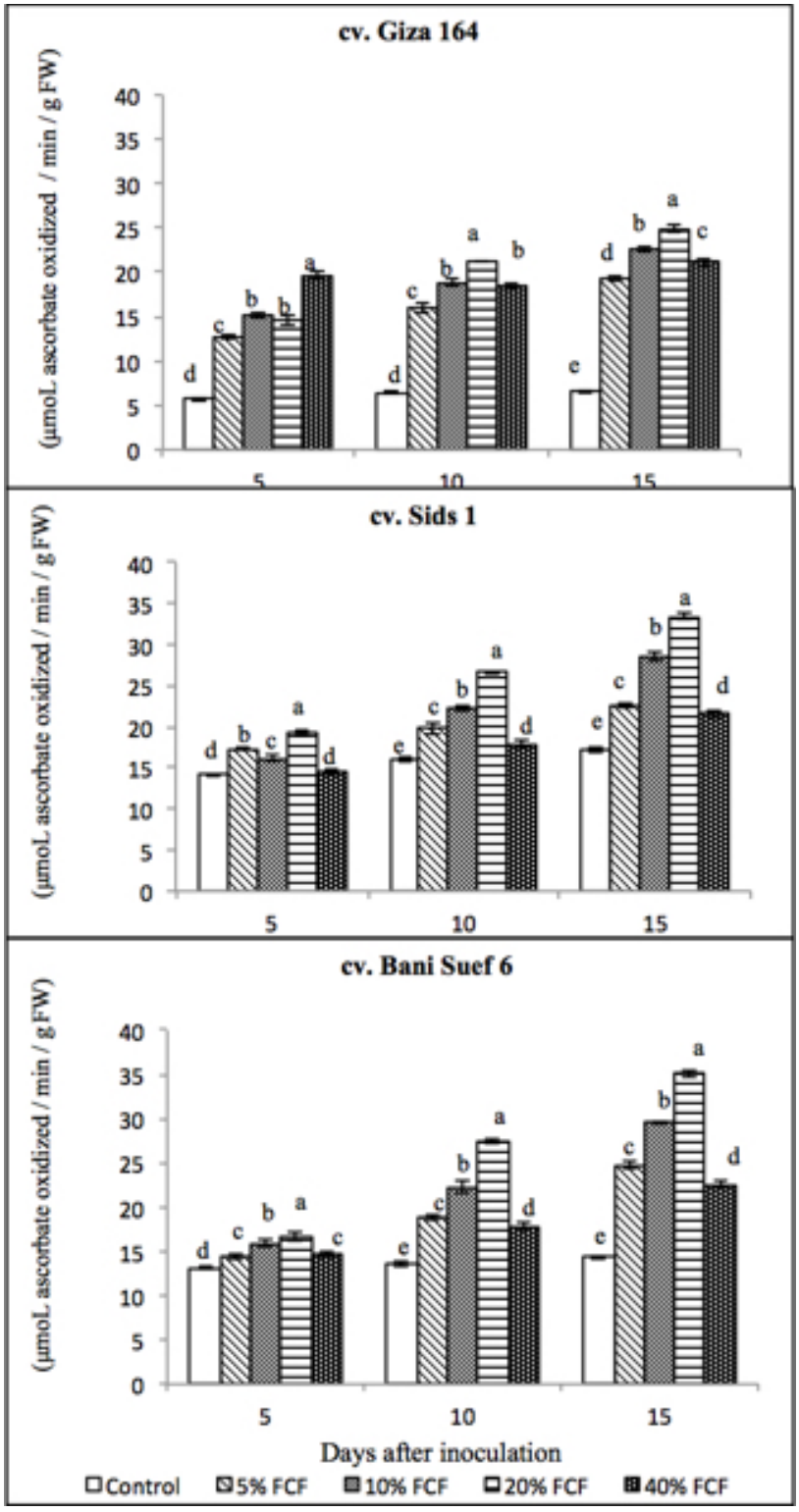

Figure 3. Effect of different concentrations of *F. graminearum* culture filtrate on ascorbate peroxidases activity of shoots of wheat (*Triticium aestivium* L.) cv. Giza 164, cv. Sids 1 and (*Triticium durum* L.) cv. Bani Suef 6 at 5, 10 and15 days after inoculation. Data are means of three samples ± SD. Within different time, different letters indicate significant variation according to Duncan's multiple range test at P = 0.05. FCF: Fungal cultures filtrate.

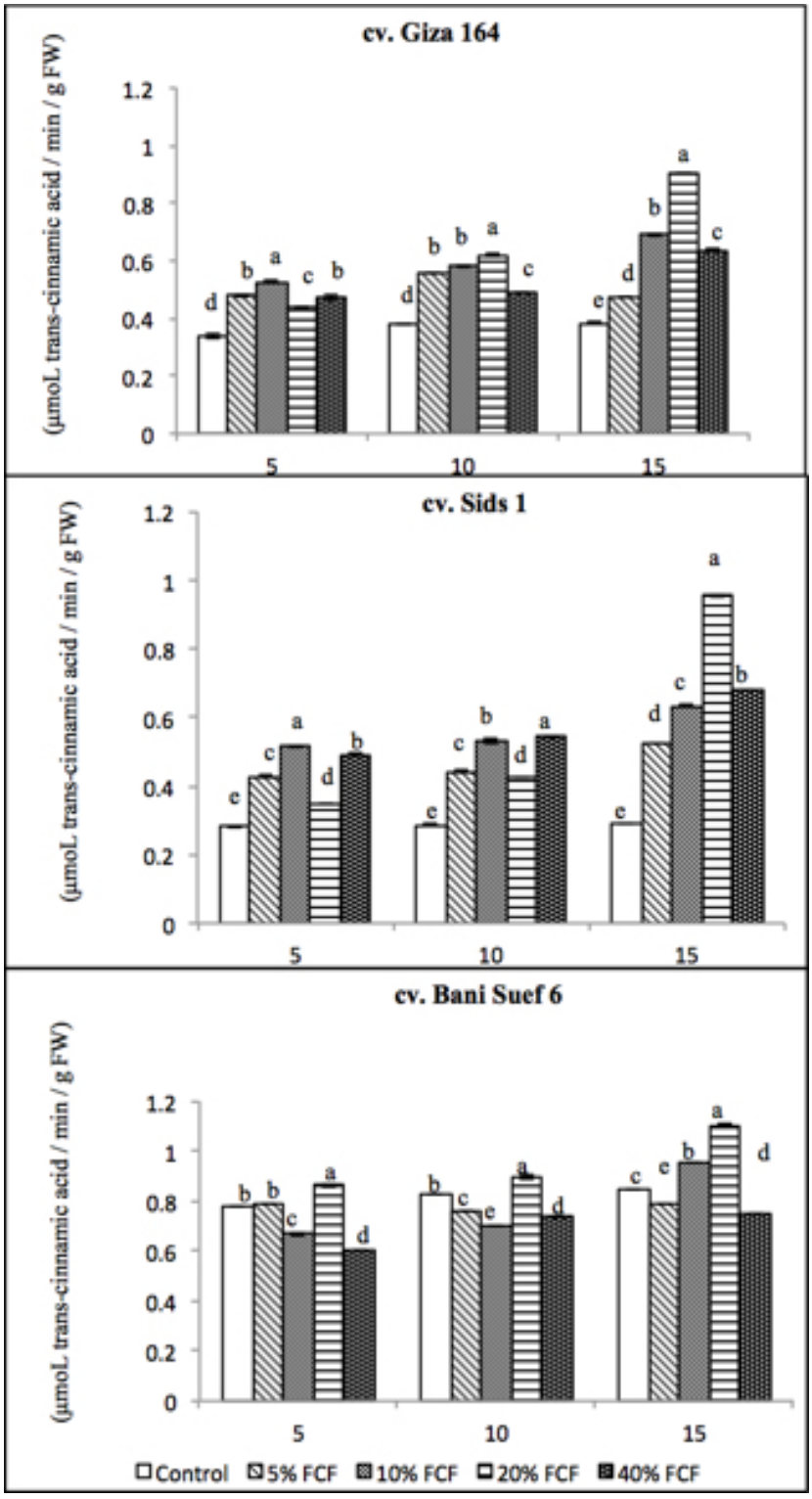

Figure 4. Effect of different concentrations of *F. graminearum* culture filtrate on phenylalanine ammonia lyase activity of shoots of wheat (*Triticium aestivium* L.) cv. Giza 164, cv. Sids 1 and (*Triticium durum* L.) cv. Bani Suef 6 at 5, 10 and 15 days after inoculation. Data are means of three samples ± SD. Within different time, different letters indicate significant variation according to Duncan's multiple range test at P = 0.05. FCF: Fungal cultures filtrate.

DISCUSSION

Changes in protein. The present results showed that total soluble protein contents in wheat shoots were decreased in cv. Giza 164, but increased in cv. Sids 1 and cv. Bani Suef 6 plants inoculated with different concentrations of *Fusarium graminearum* compared with non-inoculated plants. The concentration 10% FCF caused the highest reduction in soluble protein content (41.14%) in shoot of cv. Giza 164 after five days from inoculation as shown in Table (1). Some authors worked on plants subjected to stress also recorded these results. Vafaii *et al.* (2013) stated that *Fusarium culmorum* exhibited significant reduction in protein content of wheat leaf tissues as a result of pathogen infection. They explained this reduction to some activities related to a hypersensitive response. Arad and Rechimond (1976) attributed the decrease in protein synthesis to an increase in RNase under abiotic stress. While Egorov (1985) ascribed it to the inhibition of amino acid incorporation into protein at the final stages of the process without affecting the initial stage of activation of amino acids.

On the other hand, all concentrations of fungal culture filtrate (FCF) significantly increased the protein content in two wheat cultivars (cv. Sids 1 and cv. Bani Suef 6) reaching the maximum value at 20% of FCF, thereafter, it was decrease gradually at 40% of FCF as shown in Tables (1). The increased protein in treated plants might be responsible for defense response in plants. After the contact with the pathogen, a series of peptides and proteins are expressed and many of these compounds present have direct antimicrobial properties. Plant defense-related genes are known to be activated by the generation of endogenous plant signal molecules following the primary recognition of pathogen-encoded elicitors (Maffei *et al.,* 2007). Boller (1985) mentioned that proteins are associated with the defense of plants against fungi and bacteria by their action on the cell walls during invasion. These proteins are referred to as pathogenesis related (PR) proteins; some of these have enzymatic activities, while others have no clearly defined functions (Van Loon, 1999; Mishra *et al*., 2011). In this respect, the current results suggested that wheat cultivars Sids 1 and Bani Suef 6 are more tolerant to biotic stress than cultivar Giza 164 due to their higher protein contents.

Changes in antioxidant enzymes. In the present study, catalase activity was increased gradually in shoots of the three wheat cultivars Giza 164, Sids 1 and Bani Suef 6 inoculated with different concentrations of *F. graminearum* extract. The maximum value of catalase activity was recorded at 20% of *F. graminearum* extract after 15 days from inoculation reaching 102.48%, 132.86% and 112.02% in cv. Giza 164, cv. Sids 1 and cv. Bani Suef 6, respectively as shown in Figure (1). These results are in agreement with those of Kwon and Anderson (2001), who mentioned that superoxide dismutase and catalase isozymes activity were enhanced in the wheat leaves infected with *Fusarium*. Anand *et al.* (2009) confirmed that phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO) and catalase (CAT) activities contributed to higher resistance in green chilli fruits against *Colletotrichum capsici* and *Alternaria alternate.* Aslo, Hanfei *et al.* (2013) stated that the level antioxidant defensive enzymes (POX, PPO, SOD and CAT) increased in melon seedlings in response to infection by *Fusarium oxysporum f. sp. Melonis* Race 1.2. In the present study cultivar Sids 1 was found to has

the highest CAT value in inoculated plants compared to control.

The host cell under pathogenesis might accelerate the terminal respiratory pathway, which may lead to increase in CAT activity. Moreover, Anand *et al.* (2009) reported that an increase of CAT activity in the inoculated fruits were due to an increased H_2O_2 in the host tissues. Catalase in defensive mechanisms against pathogen can prevent the oxidative damage through rapid removal of H_2O_2.

In the present study, POX activity also was higher in inoculated shoots of the three wheat cultivars (Giza 164, Sids 1 and Bani Suef 6) compared to the corresponding non-inoculated ones with Sids 1 cultivar with the highest increase in POX compared to control (Figure 2). Such results are in consistent with the findings of Mohammadi and Kazemi (2002) who observed an increase in POX activity in susceptible wheat plants (cv. Falat) after inoculation with *F. graminearium* in the milk stage. Arfaoui *et al.* (2005) found that pretreatment of chickpea with *Rhizobium* increased significantly the level of peroxidase, polyphenol oxidase and total phenolics. El- Khallal (2007) concluded that POX activity was significantly increased in tomato plants in response to infection with *F. oxysporum*. The observed increase in both CAT and POX activities in cultivar Sids 1 might contribute to increased tolerance in response to inoculation with *F. graminearium* compared to the other cultivars.

In addition, Cherif *et al.* (2007) reported that a significant increase in POX specific activity in resistant and susceptible wheat cultivars following the inoculation with *F. graminearium* conidia. Ojha and Chatterjee (2012) stated that both polyphenol oxidase and peroxidase activities were found to be much higher in tomato plants infected with *F. oxysporum f. sp. Lycopersici* and gradually increased as the infection period progressed.

Peroxidase activity was implicated in a number of diverse phenomena observed in plants such as lignification, suberization, cell elongation, growth and regulation of cell wall biosynthesis and plasticity, which diversified during disease period (Anjum *et al.* 2012).

The results obtained from this study revealed that ascorbate peroxidase plays a key role in eradicating hydrogen peroxidase molecules. From Figure (3), APX activity was significantly increased in the shoots of wheat cultivars that inoculated with different concentrations of FCF reached the maximum at 20%, thereafter, it decreased in the three wheat cultivars at 40% of FCF through the experimental period. This study was in consistent with that obtained by Mandal *et al.* (2009) who studied the biochemical characteristics of the oxidative burst during interaction between *Solanum lycopersicum* and *F. oxysporum* f. sp lycopersici. They reported that activities of antioxidative enzyme (SOD, CAT, POX and APX) increased in response to pathogen inoculation. A major hydrogen peroxide detoxifying system in plant cells under stress is the ascorbate-glutathione cycle, in which ascorbate peroxidase (APX) isoenzymes convert H_2O_2 into H_2O, using ascorbate as a specific electron donor, particularly in the chloroplast (Gherbawy *et al*., 2012). The maximum increase in APX activity, in response to treatment with FCF, was detected in cultivar Giza 164 while the lowest increase was detected in cultivar Sids 1 indicating that the defense mechanism in wheat cultivars differ where Sids 1 depends on CAT and POX enzymes as a detoxification agents Giza 164 depends on APX

enzyme.

Changes in PAL activity. The results in Table (2) showed an increase in PAL activity in bread wheat cultivars (Giza 164 and Sids 1) at all FCF concentrations, while the PAL activity was decreased in durum wheat cultivar (Bani Suef 6) in all FCF concentrations except at 20% of FCF. In line with our results, Anand *et al.* (2009) demonstrated that PAL activity increased both in ripe and green chilli fruits in response to inoculation with the fruit rot pathogen. Stadnik and Buchenauer (2000) also reported the enhancement of PAL activity and accumulation of cell wall-bound phenolic compounds in wheat plants in response to powdery mildew infection. In contrast, Vafaii *et al.* (2013) stated that PAL activity significantly reduced in wheat plants after inoculated with *F. culmorum* compared with uninoculated plants. However, with prolonged pathogenic stress, the PAL levels were lower compared with other treatments or control. The chickpea seedlings exposed to cell wall protein of *F. oxysporum* f. sp. *ciceri*, showed enhanced synthesis of phenols, pathogenesis-related proteins and activities of PAL and peroxidase relative to water treated controls (Saikia *et al.* 2006).

PAL activity could be induced in plant pathogen interactions. PAL, one of the key enzymes in the phenylpropanoid pathway and the flavonoid pathway, was increased in both incompatible and compatible interaction between plants and pathogens (Harllen *et al*., 2004). This enzyme has been correlated with defense against pathogen in several plants including tomato (Borden and Higgins, 2002), pepper (Jung *et al*., 2004) and wheat (Mohammadi and Kazami, 2002).

Changes in proline content. The data presented in Table (3) demonstrated that proline content in the three wheat cultivars significantly increased in response to inoculation with different concentrations of FCF. In this respect, Gherbawy *et al.* (2012) indicated that wheat plants inoculated with *F. culmorum*, *F. graminearum* and *F. sambucinum* as well as a mixture of all the *Fusarium* species exhibited an increase in proline content. Meanwhile, Ashry and Mohamed (2011) observed that proline markedly increased in resistant and susceptible flax lines during infection with powdery mildew as compared to their parents. Moreover, Vafaii *et al*. (2013) mentioned that the proline content in wheat plants that inoculated with *F. culmorum* was higher than non-inoculated ones.

Free proline accumulates in plants in response to the imposition of a wide range of biotic and abiotic stresses (Hare and Cress, 1997). Accumulation of the amino acid proline in the present work is one of the indications to plant adaptation to unfavorable conditions. This increment might be attributed to the cell defense reactions. The physiological significance of proline accumulation has been assigned a role as a cyto-solute, as a convenient source of energy, as a protective agent for cytoplasmic enzymes. Proline regulates the osmotic equilibrium between the cytoplasm and vacuole. Proline can serve as a protector of enzyme denaturation, as a reservoir of nitrogen and carbon or as stabilizer of the machinery for protein synthesis (Maiti *et al.,* 2009).

Changes in phenol content. The results (Table 4) indicated that phenol content slightly changed in the three wheat cultivars inoculated with different concentrations of FCF after five days from inoculation, while at ten and

fifteen days, the phenol content significantly increased in inoculated three wheat cultivars with different concentrations of FCF. The results obtained in the present work agree with those of Anjum *et al.* (2012) who found that infection induced accumulation of phenolic acids in various parts of wheat plant infected with *Ustilago tritici* and total phenolic content following the progress of the disease.

The first stage of defense mechanism involve rapid accumulation of phenols at the infection site which resistance or slowing the growth of the pathogens. Ojha and Chatterjee (2012) observed that the phenol content was significantly higher in *F. oxysporum* infected tomato plants treated with either salicylic acid or *Trichoderma harzianum*. The accumulation of phenols in the infected tissue might come from the surrounding healthy leaves in order to resist the advancement of the pathogen towards the other healthy cells. Giza 164 was the cultivar with the highest phenol content compared to control. Several changes in the metabolism of the diseased plant accompany the increase in respiration after inoculation. These increases accompanied by increased activities of the pentose phosphate pathway, which is the main source of phenolic compounds (Agrios, 1997).

Worth to mention that there was a lag time of 10 days (at least) between the increase in PAL activities in inoculated cultivars Giza 164 and Sids 1 and the increase in phenolic content of these cultivars. PAL is a key enzyme in the biosynthesis of phenyl propane unit, which is a component of phenolic acids, flavonoids and lignins. The metabolism of phenolic compounds is regulated by the activity of various enzymes. The first step in biosynthesis of the phenylpropanoid skeleton in higher plants is the deamination of L-phenylalanine to yield trans-cinnmamic acid and ammonia, a reaction catalyzed by PAL (Rosler *et al*., 1997).

Changes in Flavonoids content. The data of the present study showed that the highest level of flavonoid content was recorded at 20% of FCF in the three wheat cultivars (Giza 164, Sids 1 and Bani Suef 6) as shown in Table (4). This study was consistent with that reported by Busko *et al.* (2014) who recorded that the highest concentration of flavonoid was found in winter wheat cultivars inoculated with *F. culmorum* while the level of flavonoid in control and fungicide-treated plants were significantly lower than inoculated ones.

Ma *et al.* (2014) stated also that drought stress significantly increased the total phenolic, total flavonoids, anthocyanin and schaftoside contents in wheat leaves. These results indicate that flavonoids have a protective role due to the special structure of flavonoids, e.g. hydroxyl group, double carbon bonds and modifications like glycosylation and methylation. In several plant pathogen interactions, the defense response has been associated with accumulation of flavonoids, phenolic compounds and phytoalexin. Flavonoids, a class of low molecular weight phenolic compounds, play important roles in plants, such as UV protection, as defense against pathogens and pests, as signaling with microorganisms, as auxin transport regulation and pigmentation (Winkel-Shirley, 2001).

Strong difference in expression between two maize genotypes were observed in secondary metabolism category pathway related to shikimate, lignin, flavonoid and terpenoid biosynthesis were strongly represented and induced in the resistant genotype, indicating that selection

to enhance these trait is an additional strategy for improving resistance against *F. verticillioids* infection. Taken together, the current results suggested that wheat cultivar Sids 1 is more tolerant to *F. graminearum* inoculation as evidenced by both increased activities of CAT, POX and PAL and elevated levels of proteins and flavonoids content compared to other cultivars under investigation.

CONCLUSION

In conclusion our results highlight the induced defense mechanisms in three wheat cultivars in response to *F. graminearum* infection. Infected cultivars showed increased activities of antioxidant enzymes (catalase, peroxidase and ascorbate peroxidase) and elevated levels of proline, total phenols and flavonoids content as compared with non-infected plants. On the other hand, PAL activity increased only in infected Giza 164 and Sids 1 cultivars whereas total soluble proteins increased only in infected cultivars Sids 1 and Banisuef 6. In addition, cultivar Sids 1 depends mainly on CAT and POX enzymes as detoxification agents while cultivar Giza 164 depends mainly on APX enzyme. These indicated that in addition to the common protective mechanisms detected in all the infected cultivars there are cultivar – dependent physiological changes exhibited by wheat plant during abiotic stress. In addition, our results showed that sids 1 cultivar are more tolerant to FHB than Giza 164 and Bani Suef 6 cultivars as evidenced by qualitative differences at the physiological level. These results are expected to be valuable in breeding program to produce wheat cultivars with increase biotic tolerance.

REFERENCES

Abdel-Fattah, G.M. and Asrar, A.A. (2012) *Arbuscular mycorrhizal* fungal application to improve growth and tolerance of wheat (*Triticum aestivum* L.) plants grown in saline soil. *Acta Physiol Plant.,* **34**, 267–277.

Abeysinghe, D.C., Li, X., Sun, C., Zhang, W., Zhou, C. and Chen, K. (2007) Bioactive compounds and antioxidant capacities in different edible tissues of citrus fruit of four species. *Food Chem*., **104**, 1338-1344.

Aebi, H. (1984) Catalase *in vitro*. *Methods in Enzymology* **105**, 121–126.

Agrios, G.N. (1997) Plant Pathology, 4th ed. San Diego: Academic Press, pp: 93-114.

Anand, T., Bhaskaran, R., Raguchander, T., Samiyappan, R., Prakasam, V. and Gopalakrishnan, C. (2009) Defence responses of chilli fruits to *Colletotrichum capsici* and *Alternaria alternate. Biol Plant.,* **53(3)**, 553-559.

Anjum, T., Fatima, S. and Amjad, S. (2012) Physiological changes in wheat during development of loose smut. *Trop Plant Pathol*., **37(2)**, 102-107.

Arad, S.M. and Richmond, A.E. (1976) leaf cell water and enzyme activity. *Plant Physiol*., **57(4)**, 656-658.

Arfaoui, A., Sifi, B., El Hassni, M., El Hadrami, I., Boudabbous, A. and Chérif, M. (2005) Biochemical analysis of chickpea protection against Fusarium wilt afforded by two Rhizobium isolates. *J Plant Pathol*., **4**, 35-42.

Asada, K. (1992) Ascorbate peroxidase, a hydrogen peroxide scavenging enzyme in plants. *Physiol*

Plant., **85**, 235–241.

Ashry, N.A. and Mohamed, H.I. (2011) Impact of secondary metabolites and related enzymes in flax resistance and/or susceptibility to powdery mildew. *World J Agri Sci*., **7(1)**, 78-85.

Bagdi, D.L. and Shaw, B.P. (2013) Analysis of proline metabolic enzymes in *Oryza sativa* under NaCl stress. *J Environ Biol*., **34**, 677-681.

Bates, L.S., Waldren, R.P. and Teare, L.D. (1973) Rapid determination of free proline for water stress studies. *Plant Soil*, **39**, 205-207.

Boller, I. (1985) Induction of hydrolases as a defense reaction against pathogen. In: Cellular and Molecular Biology of Plant stress, Key, J.L. and T. Kosuge (eds). Liss publisher, NewYork, pp: 247-262.

Borden, S. and Higgins, V.J. (2002) Hydrogen peroxide plays a critical role in the defense response of tomato to *Cladosporicem fulvum*. *Physiol Mol Plant Patholl* ., **61**, 227–236.

Boubakri, H., Poutaraud, A., Wahab, M., Clayeux, C., Baltenweck-Guyot, R., Steyer, D., Marcic, C., Mliki, A., Soustre-Gacougnolle, I. (2013) Thiamine modulates metabolism of the phenylpropanoid pathway leading to enhanced resistance to *Plasmopara viticola* in grapevine. *BMC Plant Biol.,* **2013**, 13-31

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. *Anal Biochem*., **38**, 248-252.

Busko, M., Goral, T., Ostrowska, A., Matysiak, A., Walentyn-Goral, D. and Perkowski, J. (2014) The effect of Fusarium inoculation and fungicide application on concentration of flavonoids (Apigenin, Kaempferol, Luteolin, Naringenin, Quercetin, Rutin, Vitexin) in winter wheat cultivars. *Amer J Plant Sci*., **5**, 3727-3736.

Chérif, M., Arfaoui, A. and Rhaiem, A. (2007) Phenolic compounds and their role in bio-control and resistance of chickpea to fungal pathogenic attacks. *Tunis J Plant Prot*., **2(1)**, 7-21.

De Gara, L., Pinto, M.C., Tommasi, F. (2003) The antioxidant systems vis-à-vis reactive oxygen species during plant–pathogen interaction. *Plant Physiol Biochem*., **41**, 863–870.

Dickerson, D.P., Pascholati, S.F., Haagerman, A.E., Butler, L.G. and Nicholson, R.L. (1984) Phenylalanine ammonialyase and hydroxy 1 cinnamate: CoA ligase in maize mesocotyls inoculated with *Helmintho sporium maydis* or *Helminthosporium carbonum*. *Physiol Plant Pathol*., **25**, 111-123.

Egorov, N.S. (1985) Antibiotics- A Scientific Approach. MIR Publishers, Moscow, pp. 374-377.

El- Khallal, S.M. (2007) Induction and modulation of resistance in tomato plants against *Fusarium* wilt disease by bioagent fungi (*Arbuscular mycorrhiza*) and/or hormonal elicitors (Jasmonic acid & Salicylic acid): 2-Changes in the antioxidant enzymes, phenolic compounds and pathogen related- proteins. *Aust J Basic App Sci*., **1(4)**, 717-732.

Fahmy, A.H., El-Shihy, O., El-Shafey, Y.H. and Modkour, M.A. (2006) Genetic transformation of Egyptian wheat cultivar (*Triticum aestivum* L.) via biolistic

bombardment using different constructs. *Ameri - Eur J Agric Environ Sci*., **1(2)**, 58-69.

Gherbawy, Y.A., El-Tayeb, M.A., Maghraby, T.A., Shebany, Y.M. and El-Deeb, B.A. (2012) Response of antioxidant enzymes and some metabolic activities in wheat to *Fusarium* spp. Infections. *Acta Agro Hun*., **60(4)**, 319-333.

Hanfei, M., Dehghani, H. and Choukan, R. (2013) The role of antioxidant enzymes and phenolic compounds in diseases resistance to *Fusarium oxysporum f. sp.* Melonis Race 1.2. *Int J Agro Plant Prod.,* **4(8)**, 1985-1996.

Hare, P.D. and Cress, W.A. (1997). Metabolic implications of stress-induced proline accumulation in plants. *Plant Growth Regul*., **21**, 79-102.

Harllen, S.A.S., Reginaldo, S.R., Dirceu, M., Bernardo, A.H., Maria, C.B.P. and Ann, M. (2004) Rhizobacterial induction of systemic resistance in tomato plants: Non-specific protection and increase in enzyme activities. *Biol Cont.,* **29**, 288-295.

Hung, P.V., Maeda, T. and Morita, N. (2009) Total phenolic compounds and antioxidant capacity of wheat graded flours by polishing method. *Food Res Int*., **42**, 185-190.

Jiao, F., Kawakami, A. and Nakajima, T. (2008) Effects of different carbon sources on trichothecene production and *Tri* gene expression by *Fusarium graminearum* in liquid culture. *FEMS Microbiol Lett*., **285**, 212-219.

Jung, E.H., Jung, H.W., Lee, S.C., Han, S.W., Heu, S. and Hwang, B.K. (2004) Identification of a novel pathogen-induced gene encoding a leucine-rich repeat protein expressed in phloem cells of *Capsicum annuum*. *Biochem Biophys Acta*, **1676**, 211–222.

Kazan, K., Gardiner, D.M. and Manners, J.M. (2012) On the trail of a cereal killer: recent advances in *Fusarium graminearum* pathogenomics and host resistance. *Mol Plant Pathol*., **13**, 399–413.

Kwon, S.I. and Anderson, A.J. (2001) Differential production of superoxide dismutase and catalase isozymes during infection of wheat by a *Fusarium proliferatum* –like fungal isolate. *Physiol Mol Plant Pathol*., **58**, 73-81.

Ma, D., Sun, D., Wang, C., Li, Y. and Guo, T. (2014) Expression of flavonoid biosynthesis genes and accumulation of flavonoid in wheat leaves in response to drought stress. *Plant Physiol Biochem*., **80**, 60-66.

Maffei, M.E., Mithofer, A. and Boland, W. (2007) Before gene expression: early events in plant-insect interaction. *Trends Plant Sci*., **12**, 310–316.

Maiti, R.K., Gonzalez, A.N., Ebeling, P.W., Limon, S.L.M., Avila, M.L. and Pinero, J.L.H. (2000) Proline and protein profile as indicators of resistance to biotic and abiotic stresses in Bean (*Phaseolus vulgaris L.). Rev Agric Res*., **4**, 231-237.

Mandal, S., Mallick, N. and Mitra, A. (2009) Salicylic acid induced resistance to *Fusarium oxysporum f. sp. Lycoperisici*. *Plant Physiol Biochem.,* **47**, 642-649.

Mishra, V.K., Biswas, S.K. and Rajik, M. (2011) Biochemical mechanism of resistance to *Alternaria* blight by different varieties of wheat. *Int. J. Plant Pathol*., **2(2)**, 72-80.

Mohammadi, M. and Kazemi, H. (2002) Changes in

peroxidase and polyphenol oxidase activities in susceptible and resistant wheat heads inoculated with *Fusarium graminearum* and induced resistance. *Plant Sci*., **162**, 491-498.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and Bio assays with tobacco tissue cultures. *Physiol Plant*., **15**, 473–497.

Naz, A.A. (2007) Comparative mapping of genes for plants disease resistance in wheat adanced 'backcross populations' by means of DNA markers, PhD thesis, Faculty of Agriculture, University of Bonn, Germany.

Ojha, S. and Chatterjee, N.C. (2012) Induction of resistance tomato against *Fusarium oxysporum F. SP.* Lycopersici mediated through salicylic acid and *Trichoderma harzianum*. *J Plant Prot Res.,* **52(2)**, 220-225.

Quettier, D.C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M., Cazin, J., Bailleu, F. and Trotin, F. (2000) Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *J Ethnopharmacol*, **72**, 35–42

Rajendrakumar, C.S., Reddy, B.V. and Reddy, A.R. (1994) Proline-protein interactions: protection of structural and functional integrity of M4 lactate dehydrogenase. *Biochem Biophys Res Commun.,* **201(2)**, 957-963.

Rösler, J., Krekel, F., Amrhein, N. and Schmid, J. (1997) Maize phenylalanine ammonia lyase has tyrosine ammonia lyase activity. *Plant Physiol*., **113**, 175-179.

Saikia, R., Yadav, M., Singh, B.P., Gogoim, D.K., Singh, T. and Arora, D.K. (2006) Induction of resistance in chickpea by cell wall protein of *Fusarium oxysporum* f. sp. *Ciceri* and *Macrophomina phaseolina*. *Curr Sci.,* **91(11)**, 1543-1546.

Stadnik, M.J. and Buchenauer, H. (2000) Inhibition of phenylalanine ammonialyase suppresses the resistance induced by benzothiadiazole in wheat to *Blumeria graminis* F.SP. tritici. *Physiol Mol Plant Pathol.,* **57**, 25-34.

Torres, M.A., Jonathan, D.G. and Dangl, J.L. (2006) Reactive oxygen species signaling in response to pathogen. *Plant Physiol.,* **141**, 373-378.

Vafaii, A.A., Ketabchi, S. and Moradshahi, A. (2013) Effect of Benzo (1,2,3) thiadiazole-7-Carbothioic acid and S-methyl ester (BTH) on biochemical responses of wheat seedlings infected by *Fusarium culmorum*. *Int J Farm Alli Sci.,* **2(12)**, 334-342.

Van Loon, L.C. (1999) Occurrence and properties of plant pathogenesis- related proteins. In: Datta SK, Muthukrishnan S (eds) Pathogenesis-related proteins in plants. CRC Press, Boca Raton, Fla., pp 1–19.

Velikova, V., Yordanov, I. and Edreva, A. (2000) Oxidative stress and some antioxidant system in acid rain treated bean plants: Protective role of exogenous polyamines. *Plant Sci.,* **151(1)**, 59 – 66.

Venkatesh, K.V., Kumar, K.G., Pradeepa, K., Kumar, S.R., Kumar, R.S. (2013) Biochemical markers assisted screening of *Fusarium* wilt resistant *Musa paradisiaca* (L.) cv. puttabale micropropagated clones. *Indian J Exp Biol*., **51(7)**, 531-42.

Weeks, J.T., Anderson, O.D. and Blechl, A.E. (1993)

Rapid production of multiple independent lines of fertile transgenic wheat (*Triticum aestivum* L.). *Plant Physiol.*, **102**, 1077–1084.

Winkel-Shirley, B. (2001) Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. *Plant Physiol.,* **126**, 485-493.

Zheng, H-Z, Cui Ch, I., Zhang, Y-t, Wang, D., Jing, Y. and Kim, K. Y. (2005) Active changes of lignification-related enzymes in pepper response to *Glomus intraradices* and/or *Phytophthora capsici*. *J Zhejiang Univ Sci.,* B **6(8)**, 778–786.