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Fusarium Induced Anatomical and Biochemical Alterations in Wild Type and DPA-treated Wheat Seedlings

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Abstract

Wheat (*Triticum aestivum*) employs various strategies to defend against *Fusarium oxysporum*, a soil-borne vascular fungal pathogen that disrupts structural integrity and metabolism. The purpose of this research was to ascertain the alterations of anatomical and biochemical responses in wild-type (WT) and DPA-treated wheat (*T. aestivum*) seedlings exposed to *F. oxysporum*. The WT and DPA-treated seedlings showed disorganization of parenchyma cells, sclerenchyma cells, vascular bundles (VBs), and lower numbers of xylem (Xy) and phloem (Ph) cells, and reduced thickness of the cuticle layer (C) at the epidermal layer of shoots. The content of chlorophyll (Chl), carbohydrate, and nucleic acid was reduced in WT and DPA-treated seedlings during infection. Enhanced defense responses through peroxidase (POD), and polyphenol oxidase (PPO) was observed to be high in WT as compared to DPA-treated seedlings under stress condition. In addition, the content of salicylic acid (SA) and phenolics was increased in WT than DPA under stress condition. However, the DPA-treated seedlings showed enhanced growth of fungal mycelia compared to WT during stress condition. Hence, the anatomical and biochemical aspects of DPA-treated seedlings decreased as compared to WT when exposed to *F. oxysporum*.

Keywords: *Triticum aestivum, Fusarium oxysporum,* Anatomy, PPO, SA, Phenolics

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INTRODUCTION

Plant growth and productivity are detrimentally impacted by nature's fury in the form of ecological stress components. Approximately, 96.5% of rural land areas is influenced by biotic stressors in worldwide and damaged 70% of crops.[1] Wheat (*Triticum aestivum*) is one of the world's most significant staple food crops due to its high nutritional value but often suffers from *Fusarium oxysporum* infection in many regions (Australia, China, Indonesia, Malaysia, Philippines, and India). Numerous species of *Fusarium*, such as *F. oxysporum, F. tabacinum, F. solani, F. sulphureum, F. avenaceum,* and *F. eumartii* are wheat-specific and responsible for *Fusarium* wilts.[2] Wheat has played an outstanding role in feeding a hungry world and the demand for wheat is rising worldwide due to the increasing rate of human population.[3]

F. oxysporum is a soil-borne vascular pathogen promotes wilt disease in plants. The spores of *F. oxysporum* can penetrate into the cracks or wounds at the root region.[4] Inside the root, the parenchymatous tissues are occupied by young mycelia, which invade the endodermis as well as reach at the xylem (Xy) vessels via pits. The Xy vessels are blocked due to microconidial spore, consequently lowering the transpiration rate, which causes profound cytological changes resulting in wilt symptoms.[5] The surrounding parenchyma and sclerenchyma cells changed their structure to defend the *Fusarium* proliferation and crushed the vessels in the vascular system. This induces wilting of the whole plant, which ultimately results in death.[6] In addition, the palisade parenchyma cells were observed to be smaller, and thinner leaf blades in plants during infection. It reduces photosynthesis and transpiration in leaves, and it inhibits the growth of roots and shoots.[7] A thick layer of cuticle was appeared at the upper epidermis in infected plants as compared with healthy plants.[8] Garcia-Viera et al.[9] reported that papaya ring spot virus caused histochemical changes include distorted palisade cells and disintegrated spongy cells in the leaves of papaya plants. The persistent of disease symptoms are characterized by foliar chlorosis followed by folding and stunting of plants.

Fusarium causes significant damage to crucial biological components such as lipids, proteins, DNA, and RNA,[10] stimulates the abundance of reactive oxygen species (ROS), inhibits growth, and development with tissue necrosis.[6] To neutralize the ROS-induced oxidative stress inside VBs, the plants have developed a basal defense system by the induction of salicylic acid (SA), which promotes the synthesis of phenolics that organize the secondary cell wall of internal tissue during infection,[11] and also antioxidant system, i.e., enzymatic [ascorbate peroxidase (APX), polyphenol oxidase (PPO), catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and non-enzymatic glutathione (GSH), and ascorbate (ASC)].[12] In addition, the phenol synthesizing PAL enzyme was inhibited by 2,4-DPA, which significantly reduce the level of intermediates of the phenylpropanoid pathway in *Lycopersicon esculentum*.[13] It is also reported that suppression of phenolics content induced susceptibility against fungal pathogens in wheat, tobacco, and flax plants.[14]

It is well established that *Fusarium* induced wilt disease impaired the anatomical and biochemical level of wheat seedlings due to disintegrated vascular bundles (VBs), reduced photosynthetic activity, SA, and phenolic contents.[15] Therefore, the goal of the current study was to identify the anatomical and biochemical alterations in wheat seedlings after *Fusarium* invasion.

MATERIALS AND METHODS

Plant material and growth condition

Wheat seeds (*T. aestivum*) were surface sterilized using 0.01% $HgCl_2$, followed by three times washing with sterile distilled water. In order to promote germination, the sterilized seeds were incubated in sterilized petri dishes lined with wet muslin cloth for 4-5 d at room temperature (RT). Sprouted seeds were kept in a growth chamber for 7 d at 32°C, 80% relative humidity (RH), with 16 h photoperiod (240 µmol/ m^2s) and an 8 h dark period at 26°C, 70% RH.[16] Young wheat seedlings were transported to sterilized glass test tubes containing distilled water after 7 d. One set of seedlings was cultured with 1 ml of 1 mM 2,4-DPA

suspension for 48 h at room temperature.[17] In the control set, seedlings were grown in distilled water and kept under non-stressed condition.

Pathogen inoculation

F. oxysporum was propagated in dim light at 28 ± 2°C for 4 d to produce efficient sporulation. After that, the wheat seedlings were inoculated with 4 d old *F. oxysporum* spores (1×10^6 spores ml^{-1}) by pouring the spore culture into each set of test tubes and keeping them for another 7 d for further growth and development under stressed condition.

Localization of fungal structure

The fungal structures present inside the shoot tissues were observed under light microscope after decolorized with acetic acid: ethanol: water (2:2:1) solution at room temperature and stained with lactophenol cotton blue by protocol of Charya and Garg.[18]

Anatomical studies

The decolorized shoot sections were stained with toluidine blue for viewing anatomical structures using a light microscope.[19]

Assay of photosynthetic pigments and CSI

The ice-chilled 80% acetone was used for homogenization of fresh shoot tissue (0.5 g) and centrifuged at 4500 rpm for 10 min at 4°C. The absorbance of the supernatant was read at 663, 645, and 665 nm.[20]

The chlorophyll content was estimated by utilizing the following formula:

Chl a (mg/mL) = $-1.93A_{646} + 11.93A_{663}$

Chl b (mg/mL) = $20.36A_{646} - 5.50A_{663}$

Chlorophyll a+b (Chl) (mg/mL) = $6.43A_{663} + 18.43A_{646}$

According to Sairam et al.,[21] the CSI was estimated and calculated as follows:

CSI= (Total chlorophyll under stress/Total chlorophyll under control)× 100

Assay of O_2•-

The localization of O_2•- was carried out histochemically in shoot samples using the protocol outlined by Pyngrope et al.[22] The shoot tissue samples were dipped in a sterilized glass beaker containing 6 mM Nitro Blue Tetrazolium Chloride (NBT) and 10 mM sodium azide solution (1 mg ml^{-1}, pH-7.5) for 12 h in the dark at RT. The leaf samples were again dipped in decolorizer reagent and boiled for 5 min to decolorize them. After the decolorization, the localized O_2•- was visualized as purple spots. In addition, the O_2•- content was also calculated using the protocol of Pyngrope et al.[22] The shoot tissues were dipped in 3 ml of a 10 mM K_3PO_4 buffer (pH-7.8) comprising 0.05% NBT and 10 mM sodium azide for 1 h. After that the extract was boiled for 15 min at 85°C. The absorbance was recorded at 580 nm and expressed in µmol g^{-1} f.w.

Assay of carbohydrates content

The total sugar content was measured using the protocol of Verma et al.[23] The ethanol (1ml) extract of shoot tissue was mixed with 4 ml of anthrone reagent (cold). Then the extract was boiled for 10 min and the absorbance was taken at 620 nm with expressed in mg g^{-1} f.w. In addition, the reducing sugar content was determined by the protocol of Afzal et al.[24] In a similar manner, the ethanol extract (3 ml) of shoot tissue was added to 3 ml of 3,5-dinitro-salicylic acid (DNSA) solution. The mixture was heated for 5 min and 1 ml of a 40% sodium potassium tartrate solution was added for stabilization. After cooling, the content was read at 515 nm and denoted in mg g^{-1} f.w. Moreover, the content of non-reducing sugar was estimated by deducting the reducing sugars from the total soluble sugar content. According to Parida et al.,[25] the starch content was determined by mixing the residues of alcoholic extracts of the shoot tissues with 5 ml of 52% perchloric acid (PCA) for 1 h. 1 ml of the PCA extract was added to 3 ml of anthrone solution. The PCA mixtures were boiled for 7.5 min and then read the color intensity at 630 nm. The starch content was estimated from the standard curve of glucose in a range of 0-100 µg ml^{-1} and denoted as mg g^{-1} f.w.

Assay of nucleic acid content

DNA was estimated following the method described by Lajmi.[26] The nucleic acid extract (1.5 ml) of each shoot tissues was mixed with diphenylamine solution (0.3 ml) separately, and kept in water bath at 55°C for 15 min. Then the absorbance of the extracts was recorded at 610

nm using a standard curve for standard DNA and denoted in mg g^{-1} f.w. Similarly, the RNA was also estimated adopting the technique of Lajmi.[26] The nucleic acid extract (1.5 ml) was mixed with 3 ml of orcinol reagent. Then the extracts were boiled at 55°C for 20 min and the absorbance was read at 660 nm. The RNA content was also assessed using the assistance of a standard curve prepared with standard RNA (20 to 100 µg ml^{-1}) and denoted in mg g^{-1} f.w.

Assay of POD activity

1.0 g of fresh shoot tissue was grinded with 0.1 M K_3PO_4 buffer (pH-6.6), and the homogenates were centrifuged at 18000 rpm for 15 min at 4°C. Following Kaur et al.,[27] the enzyme extract was mixed with 3 ml of a 0.05 M pyrogallol reagent, and the mixture was then incubated at 4°C for 10 min. The reaction mixture was added to 0.5 ml of H_2O_2 (0.5%) reagent, followed by incubation for 5 min. Then the absorbance was recorded at 420 nm and expressed in mmol guaicol min^{-1} g f.w.

Assay of SA content

0.2 g of shoot tissues were homogenized with 95% ethanol and vortexed at 2500 rpm for 15 min at 4°C. The homogenates were mixed with 1 ml of 1% ferric chloride to estimate salicylic acid following the protocol of Naaz et al.[28] The absorbance of supernatants was recorded at 540 nm and expressed as mg g^{-1} f.w.

Total phenolic contents

0.5 g shoot tissues were crushed using 80% cold methanol and the extracts were vortexed at 10000 rpm at 4°C for 20 min. The reaction solution was read at 280 nm using the Folin–Ciocalteu reagent, regarding the protocol of Dai et al.[29] and estimated from the standard curve of gallic acid and denoted in mg g^{-1} f.w.

Assay of PPO activity

According to the protocol of Elsheery et al.,[30] PPO activity was measured by the homogenization of 0.5 g of shoot tissue in 2 ml of 50 mM Tris-HCl (including 0.4 M sorbitol and 10 mM NaCl) (pH-7.2). The extracts were vortexed at 2000 rpm for 10 min, followed by mixing with 2.5 ml of 0.1 M K_3PO_4 buffer (pH-6.5) and 0.3 ml of catechol reagent. The absorbance was recorded at 495 nm for every 30 sec up to 5 min and expressed as U g^{-1} f.w. The equation below can be used to determine the activity of PPO.

Enzyme units in the sample = K (ΔA/min)
where, K for catechol oxidase = 0.272

Statistical analysis

For the various parameters of WT and DPA seedlings, values are shown as the

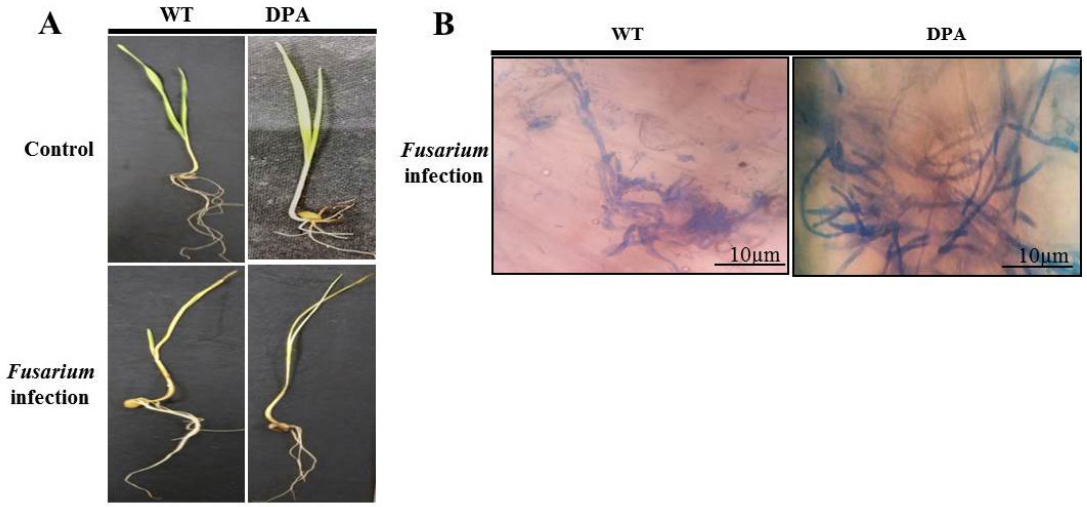

Figure 1. Plant phenotype and fungal growth A. WT and DPA under control and *Fusarium* infection, B. Mycelial growth

mean of three replicates. Here, the average of three replicates denotes the "mean of three independent seedlings," following the Student's t-test. Significance was defined as $p \leq 0.05$ (*). Data was given as mean ± standard error of mean (SEM). The graph was performed using the GraphPad Prism software.

RESULTS

Morphological and anatomical analysis of WT and DPA shoots under stress condition

The leaves' surface area shrunk, their edges curled inward, and they wilted and became yellow due to chlorosis and stunted growth in DPA, while all these symptoms observed to be less in WT when exposed to *F. oxysporum*. However, the WT and DPA exhibited less vigor and growth under the stress condition than under the non-stress condition (Figure 1A).In another investigation, the lactophenol cotton blue staining of shoots displayed fungal mycelial pattern present within the infected seedlings. An enhanced accumulation of fungal mycelia was found in DPA, which is indicative of high disease severity (Figure 1B).

The internal structure of the wheat shoot organ was studied under stress condition, which indicates the severity of damage at the tissue level. Figure 2A revealed that the disintegrated and reduced VBs in WT and DPA under stress condition compared to non-stress condition. In addition, the mechanical tissue like sclerenchyma

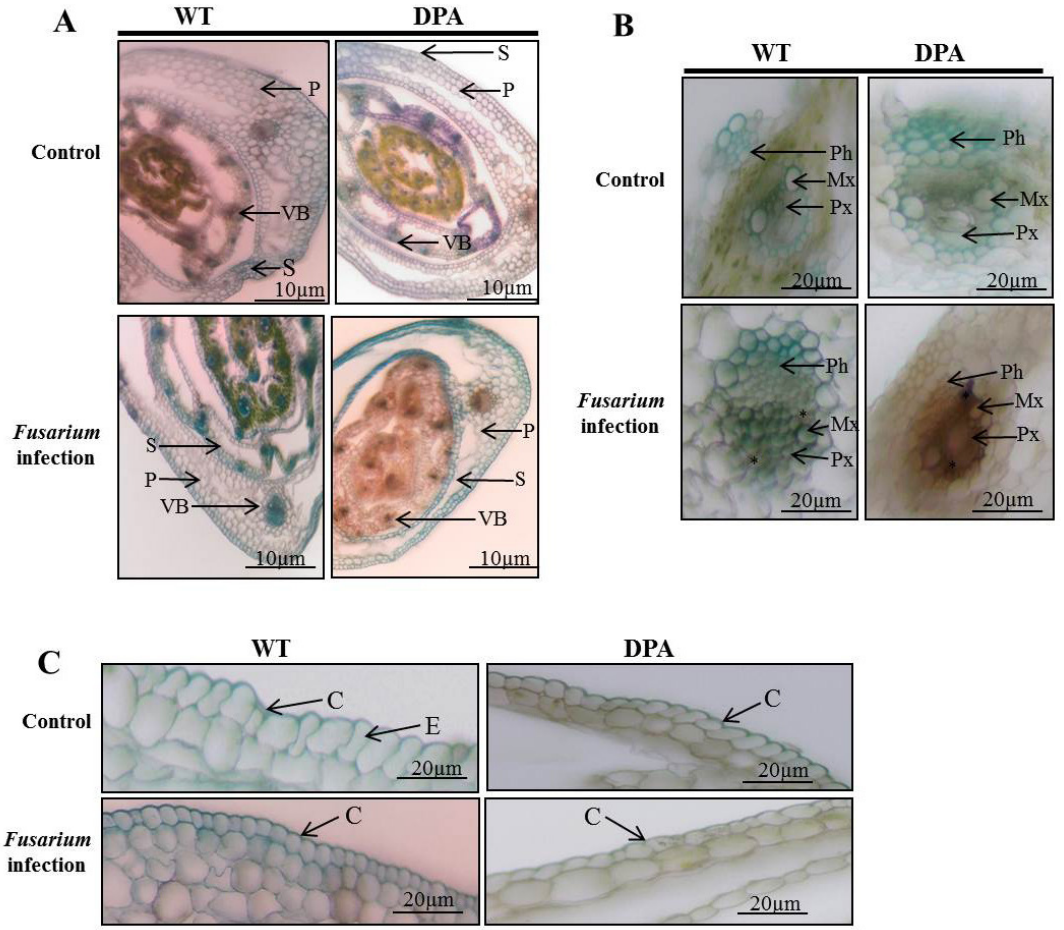

Figure 2. Histological analysis of internal structure A. TS of shoot, B. VBs, C. Epidermal layer. * indicate the amorphous materials Abbreviations: P- parenchyma; VB- vascular bundle; S- sclerenchyma; Ph- phloem; Mx- metaxylem; Px- protoxylem; C-cuticle; E-epidermis.

was conferred resistance against fungal infection due to its thickened lignified cell wall. The blue-green colored sclerenchyma cells were increased in WT as compared to DPA. Moreover, the parenchyma cells were disorganized more in DPA than WT in response to stress condition. The Xy

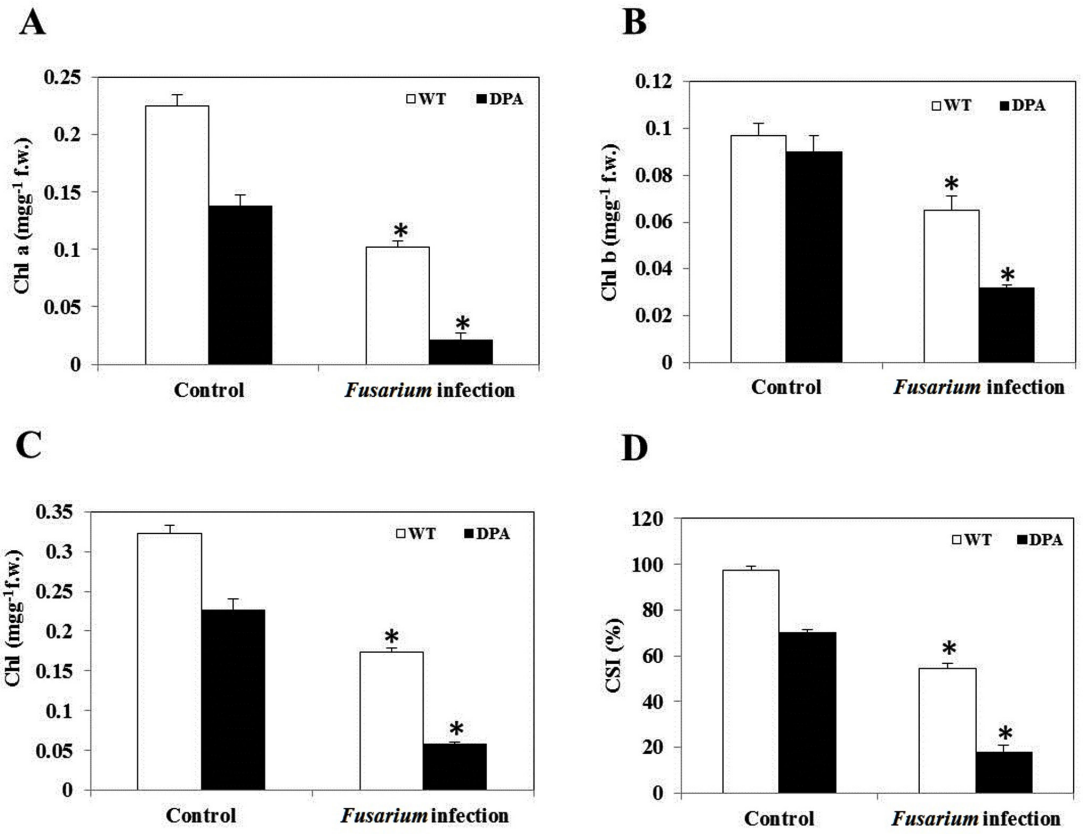

Figure 3. Photosynthetic pigments A. Chl a, B. Chl b, C. Chl, D. CSI (%)

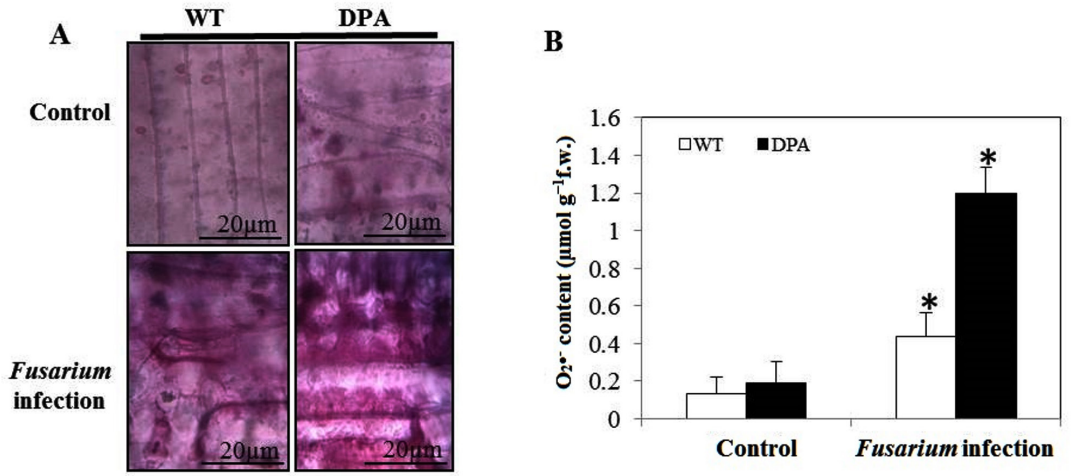

Figure 4. *In vitro* formation of O_2•- A. Localization of O_2•- B. O_2•- content

and phloem (Ph) are the conducting tissues, which translocate nutrients from the source to sink cells. Under stress condition, the numbers of Xy (green) and Ph (red) cells were lower in DPA than WT. The metaxylem (Mx) and protoxylem (Px) were studied under stress condition to learn about the water transportation process, which reflects the metabolic activity including photosynthesis and transpiration in the shoot. The Mx, and Px were observed to be disintegrated in DPA than WT during stress condition. In addition, some amorphous materials were deposited in WT and DPA under the stress condition (Figure 2B). In another investigation, a protective cutin and wax-made cuticle layer was studied for its inhibitory role against fungal invasion under stress condition. The cuticle layer of the epidermis increased in WT but decreased in DPA (Figure 2C).

Photosynthetic pigments and their stability during stress condition

Chlorophyll (Chl) is a photosynthetic component that is utilized in the light-harvesting metabolic process, including the light reaction and

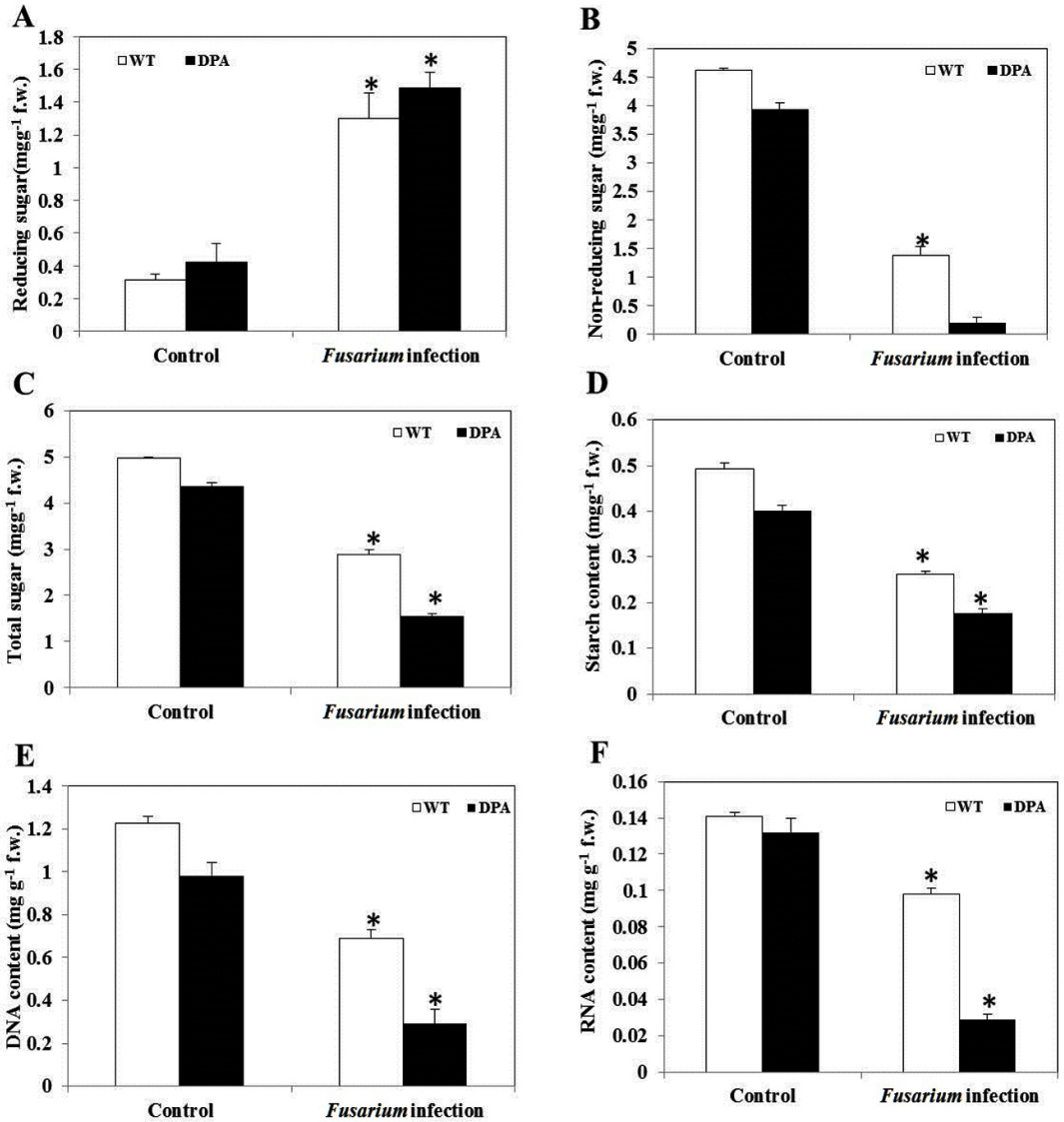

Figure 5. Macromolecules content A. Reducing sugar, B. Non-reducing sugar, C. Total sugar, D. Starch, E. DNA, and F.RNA

the Calvin cycle. It converts inorganic compounds into organic compounds. *Fusarium*-induced oxidative stress caused a decrease in chl content under stress condition. The Chl a was decreased by 1.64-and 3.5-fold in WT and DPA (Figure 3A), whereas the Chl b decreased by 1.9- and 0.48-fold in WT and DPA under the stress condition as compared to the non-stress condition (Figure 3B). Overall, the Chl content decreased 2.4- and 1.4- fold in WT, and DPA under stress condition (Figure 3C). The potential of plants to resist stress is assessed by the chlorophyll stability index (CSI). A low CSI value means that the photosynthetic system was affected by stress. Our results showed that the CSI was decreased by 1.6- and 4.6-fold in WT and DPA under the stress condition, respectively, compared to the non-stress condition (Figure 3D).

Accumulation of O_2•- in response to *Fusarium* infection

The O_2•- content, an indicator of oxidative damage due to imbalance of redox system, was estimated in both WT and DPA exposed to *Fusarium* infection. No significant visual difference was seen in the accumulation of O_2•- in WT and DPA under non-stress condition. The DPA showed increased accumulation of O_2•- as dark blue-purpled spots as compared to WT under the stress condition (Figure 4A). Furthermore, the O_2•- content was increased by 4.0- and 6.0-fold in WT and DPA (Figure 4B).

Photoassimilates and nucleic acid content in infected seedlings

Carbohydrates are the product of the photosynthesis process. It has a crucial role in the formation of organic compounds, serves as a building block for cell wall synthesis and different metabolic pathways, and also acts as a storage food material found in parenchymatous tissues. Changes in the total sugar content in the shoot of wheat under *Fusarium*-induced oxidative stress were examined. When compared to both conditions, WT and DPA showed an increased reducing sugar content of 5.2 and 4.2-fold,

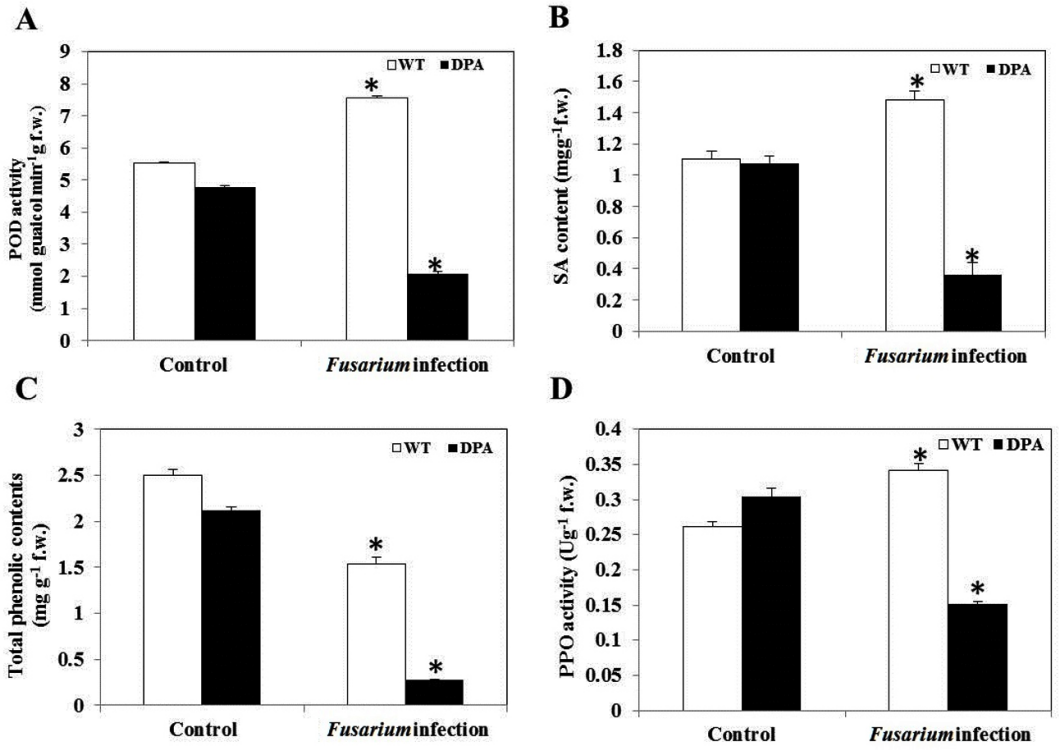

Figure 6. Secondary metabolites and antioxidant enzyme activity A. POD, B. SA, C. Total phenolics, D. PPO

respectively (Figure 5A), whereas non-reducing sugar decreased in WT and DPA by 3.1- and 9.75-fold under the stress condition (Figure 5B). In addition, the total sugar content was decreased in WT and DPA by 1.56- and 2.93-fold, respectively, in the stress condition (Figure 5C). In another investigation, the starch content was reduced to 1.68 and 1.9-fold in WT and DPA under stress condition (Figure 5D).

DNA and RNA structure and function have a key role in antioxidant enzyme regulation during fungal infection.[31] The present study revealed that the DNA content decreased by 1.71- and 3.33-fold, respectively, in WT and DPA under stress conditions as compared to the non-stress condition (Figure 5E), due to the high rate of DNA degradation during pathogen infection.[32] Moreover, the DNA repair genes were modified during infection by promoting DNA instability in plants.[33] Furthermore, the RNA content was decreased by 1.4- and 1.3-fold in WT and DPA under stress conditions (Figure 5F).

Level of SA and phenolics in WT compared to DPA during *Fusarium* infection

SA is a naturally occurring defensive phenolic compound that alleviates a high level of oxidative stress. Under *Fusarium* infection, plants lower ROS by inducing SA for proper operation, which is accompanied by a considerable rise in the activities of antioxidant enzymes, i.e., PPO and POD, under stress condition.[34] The POD activity was increased by 1.45-fold in WT and decreased by 2.4-fold in DPA, respectively, under the stress condition (Figure 6A). In addition, there was no remarkable difference in the specific content of SA in WT and DPA under non-stress condition. However, a significant increase of 1.36-fold occurred in WT in contrast to DPA, where the reduction was 3.3-fold in response to infection (Figure 6B). In addition, the phenolics decreased by 1.6- and 6.6-fold in WT and DPA, respectively (Figure 6C). Furthermore, the WT showed an increase (1.4-fold) in PPO activity and a decrease (2.0-fold) in DPA under stress condition (Figure 6D).

DISCUSSION

Fusarium-induced wilt disease is one of the biotic stress factors that could reduce the growth and development of plants. The infection may interfere with different biosynthetic pathways and retard plant growth and yield. Various studies have reported the pathogenetic effect of exogenously applying *F. oxysporum* spores to the plants.[35] In the present study, *Fusarium* exposure exhibited more yellowing and wilting in DPA in contrast to WT. The severity of wilting occurred due to blockage of xylem vessels with progressive growth of *F. oxysporum* in leaves and shoots and reduction of Chl contents, which eventually leads to death.[36] Moreover, the enhanced fungal mycelia found in the shoots of DPA-treated wheat seedlings exposed to *Fusarium* demonstrates the system's inability to block pathogen entrance.

Many reports have stated that the anatomical features of wheat stems have steadily deteriorated due to *Fusarium* infection.[37,38] The VBs acted as a conducting system to provide sap throughout the vegetative organs of plants. The Xy is affected by the endophytic *F. oxysporum* resulting interruption at the translocation system.[39] The VBs of WT and DPA were damaged and collapsed due to fungal growth, similarly, the roots and stems of corn plants were infected by a pathogenic strain of *F. verticillioides*.[40] In addition, the sclerenchyma cells provide mechanical support to VBs through thickening primary cell walls, which further acts as a mechanical barrier against fungal invasion. The present study revealed higher numbers of sclerenchyma cells in WT as compared to DPA during *Fusarium* infection. The size and thickness of sclerenchyma cells were increased in corn plants. This can be noticed as defending plant tissues against infection by an endophytic *F. sacchari.*[41] The treatment with 2,4-D induced the reduction of sclerenchyma cells in wheat seedlings during *Fusarium* infection. Moreover, the parenchyma cells conduct most of the metabolic activity, such as the photosynthesis process, and serve as a carbohydrate storage compartment while also providing support to the vascular system. The parenchyma cells were observed to be lower in DPA than WT. Similarly, these cells were also distorted, fragmented, and collapsed in *Macrophomina phaseolina* infected mungbean plants.[42]

The Mx and Px cells are responsible for the storage and long-distance transport of sap, whereas the Ph tissue translocates soluble

sugars to all organs of the plant. The Mx, Px, and Ph cells were observed to have reduced and irregular sizes in DPA as compared to WT, due to the blockage of translocation systems. Chen et al.,[43] reported that the reduced area of Mx and Px were observed in the roots, stems and leaves of wild barley (*Hordeum brevisubulatum*) infected by a *Epichloe bromicola* pathogen, and also in infected kallar grass and faba beans,[44] and sorghum during infection.[45] In the present study, the deposition of amorphous material in the VBs indicated the response of hyphal proliferation in WT and DPA shoot tissues. Nevertheless, the DPA showed pathogen colonization and produced more amorphous materials. da Conceição et al.[46] reported the evidence of phenolic-like compounds as amorphous materials produced in rice against *Pyricularia oryzae*. In addition, the cuticle layer was thick in WT, which indicates the enhanced SA and phenolic contents in response to fungal mycelium.[47] In another investigation, DPA showed a reduced cuticle layer due to lower content of SA, phenolics, and antioxidant enzyme activity.[48]

The Chl a and Chl b content were declined in *Malus pumila* Mill. leaves, resulting in reduced photosynthetic activity during apple phytoplasma infection.[49] Our study indicated that Chl a, Chl b, and Chl were decreased in DPA as compared to WT during *Fusarium* infection.[50] Aybeke[10] have investigated that total Chl content was declined due to *Fusarium* infection in several plants due to damage to pigments, the photosystems complex, and chloroplast membranes. Further, the Chl could be harmed by O_2•- leads to progressive chlorosis, dysfunction of PSI and PSII in chloroplasts, and cell death,[51] also reduces the sugar contents in parenchymatous cells of coconut palms, maize, and papaya leaves.[52] The results indicate that the inoculation of *Fusarium* enhances the pathogenesis of wheat seedlings, affecting growth and development.

Wilt disease induces the rapid accumulation of O_2•-, which is used to measure the injuries in the plants. In the present study, O_2•- accumulation was increased in DPA than WT during *Fusarium* infection. In addition, the damaged tissues also produced more ROS during infection. Moreover, the O_2•- impart photosynthesis apparatus and reduces the level of carbohydrates (primary metabolites) that serve as the progenitor for the synthesis of secondary metabolites (such as phenolic compounds) that inhibit the entry of fungal hyphae.[13]

The reducing sugar was abundant in WT and DPA during infection; similarly, the reducing carbohydrates were produced in the susceptible cv. Widusa.[53] In contrast, the non-reducing sugar was low in WT and DPA under stress condition. The photosynthates were reduced in *Phaseolus vulgaris* plants infected with *Colletotrichum lindemuthianum* and *Uromyces appendiculatus*.[54] In many plants, *Fusarium* infection is achieved by increasing the deposition of soluble sugars.[55] These chemical substances are crucial for osmotic adjustment as well as for protecting membranes and biomolecules.[56] In contrast, the total sugar and starch content of both the WT and DPA decreased under stress condition as compared to non-stress condition. Scarpari et al.[57] stated that the production of total soluble carbohydrates was reduced in *Theobroma cacao* plants infected with *Crinipelis perniciosa*, and also in sunflower (*Helianthus annuus* L.) infected with sunflower chlorotic mottle virus,[58] and leaves and stems of infected papaya plants.[59] These results suggested that the osmotic adjustment was greater in WT than DPA, indicating the structural disorganization. of parenchyma cells. The present study revealed that DNA and RNA content were decreased in WT and DPA under stress condition, similarly reported in *Ralstonia solanacearum* infected transgenic tobacco plants,[60] and also in *Meloidogyne incognita* infected tomato plants due to increase rate of ribonuclease activity.[61] Further, Zahm et al.[62] showed 2,4-DPA induced the reduction of DNA and RNA content, leading to disorganised growth and death in etiolated soybean seedlings.

In plant species, POD and PPO are part of a sophisticated antioxidant defense system that removes ROS. These antioxidant enzymes are essential for removing these harmful substances from cells and reducing ROS-induced oxidative cell damage.[63] In the present study, POD was increased in WT, this implies the protection from oxidative damage and reduced in immunocompromised DPA during *Fusarium* infection.[64] In addition, SA acts as a signal molecule for conferring resistance against fungal attack. Our results stated that, SA was increased in WT indicating the synthesis of phenolics that minimizes the

O_2•- to provide protection from fungal infection and the DPA reduced the SA related compounds in the plants under the stress condition due to inhibition of PAL activity.[65] SA induces the phenolic compounds, which are known to be maintaining cell wall strengthening in Xy and Ph tissues. Phenolics play a prime role in lignin and pigment biosynthesis, providing structural integrity and support to plants by inhibiting fungal entry. The aggregation of the phenolic monomers were required for sclerenchyma and Xy lignification with the involvement of both PPO and POD.[32] In an investigation, the phenolic contents were increased in WT, which consistent with SA but reduced in DPA under stress condition, and concomitant to rust diseased plants.[65] Moreover, the PPO activity was enhanced in WT after pathogen colonization, which was concomitant with the oxidizing phenolic compounds in tomato plants with enhanced PPO activity against bacterial canker disease.[66] Therefore, the alternation of production of SA and phenolics induces structural disorganization which facilitate the fungal proliferation. Vago et al.[67] was also reported the pathogen-induced biochemical and anatomical changes in *Lotus tenuis*.

CONCLUSION

In conclusion, 1 mM DPA treatment significantly altered the anatomical and biochemical aspects of wheat seedlings, which resulted in an increase susceptibility against *F. oxysporum*. Thus, the increase in susceptibility may be due to the structural disorganization and low level of secondary metabolites, which serves as an innate immunity against fungal pathogens. Further studies are required to unravel the molecular mechanisms underlying wheat host susceptible to *Fusarium* attack that can be used in future to counter the susceptibility.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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