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Physiological and Biochemical Response to *Fusarium Oxysporum* **Infection in Wheat**

Abhaya Kumar Sahu¹ , Punam Kumari1* , Bhabatosh Mittra1,2

¹P.G. Department of Biosciences and Biotechnology, Fakir Mohan University, Vyasa Vihar, Balasore-756089,Odisha, India. ²MITS School of Biotechnology,Bhubaneswar-751024, Odisha, India.

I. INTRODUCTION

Wheat (*Triticum aestivum*) is one of the world's biggest significant staple food crops due to its high nutritional value but often suffers from *Fusarium oxysporum* infection in many regions (Giri et al. 2013). Numerous species of *Fusarium*, such as *F. oxysporum, F. tabacinum*, *F. solani, F. sulphureum*, *F. avenaceum,* and *F. eumartii* are typically host-specific and responsible for *Fusarium* wilts (Becher et al*.* 2013). Moreover, fungal infections have become detrimental to wheat, resulting in low yields and financial losses. The demand for wheat is rising in urbanized area of India to feed the rapidly growing human population (Chandrashekar et al. 2015). Exposure to *Fusarium* infection can lead to the damages of lipids, proteins, soluble sugars, and phenolic compounds, causes cell damage and reduced plant expansions and biomass (Li et al. 2011) due to the negative impact of various reactive oxygen species (ROS), including hydrogen peroxide (H₂O₂) and superoxide radical (O⁻₂) (Borges et al. 2014). Further, it damages chlorophyll and carotenoid pigments, causing in decreased photosystem II (PS II) performance, which directly affects the growth and physiology of plants. The photoassimilates, like sugar, serve a crucial role in controlling osmotic potential and initiating a signal transduction pathway during pathogen attack. The three main classes of non-reducing sugars (NS) which are primarily engaged in plant stress responses are fructans, raffinose family oligosaccharides (RFOs), and disaccharides (sucrose, trehalose). Sucrose is a common NS produced during normal condition and composed of the reducing sugars (RS) glucose and fructose, are generated in oxidative stress situation. It is recognized as the essential sugar in plant life due to its crucial role in stress adaptation, growth, storage, signaling, and development (Salerno and Curatti, 2003).

Plants manufacture mainly enzymatic and non-enzymatic antioxidants to mitigate ROS-induced oxidative damage. Ascorbate peroxidase (APX), catalase (CAT), Superoxide dismutase (SOD), and glutathione reductase (GR) are enzyme-based antioxidants (Asada, 1992), whereas ascorbate (ASA) and glutathione (GSH) are non-enzymatic antioxidants (Li et al. 2011). O_2 to O_2 and H_2O_2 is dismutated by the SOD, which are then reduced to H_2O and O_2 by APX, CAT, GR etc. (Asada, 1992).

Hence, understanding the mechanism of plant-pathogen interaction at cytological, molecular and biochemical level is important to develop appropriate defense strategies for crop protection and management. In this research, we assessed the responses of wheat against *Fusarium* stimulated oxidative stress.

II. MATERIALS AND METHODS

Plant growth condition and F. oxysporum inoculation

Wheat seeds (*T. aestivum*) were surface sterilized using 0.01 % HgCl₂, and then three more times washing with sterile distilled water. In order to promote germination, the sterilised seeds were incubated in sterilised 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/ m²s) and an 8 h dark period at 26 °C, 70 % RH. Young wheat seedlings were transported to sterilised glass test tubes containing distilled water after 7 d. One set of seedlings were transferred to *Fusarium* spore suspension for 7 d at RT. In a control set, seedlings were grown in distilled water and kept under non-stressed conditions.

Assay of morphological parameters

The length of leaves and roots of control and *Fusarium* infected seedlings were measured through a scale.

Assay of physiological parameters

After 7 DAI (Day after inoculation), the leaves and roots from each experimental setup were taken for determination of relative water content(RWC) using fresh weight (FW) and dry weight (DW). The RWC was calculated using the method and followed the below equation (Tahjib-Ul-Arif et al. 2018).

RWC (%)= $\frac{\text{(Fresh weight-Dry weight)} \times 100}{T}$

(Turgor weight−Dry weight)

Disease Rating (DR)

The impact of infection on seedlings was assessed as a percentage of disease rating by the protocol of Warzecha et al. (2019).

 $DR\% = 100 \times (ni \times Di)/NDmax$

Where: ni—number of plants of ith category, Di—numerical value of ith category, N—total number of plants in the sample, and Dmax maximum scale value (0–5).

Assay of photosynthetic pigments

The ice-chilled 80 % acetone was used for homogenization of fresh leaves (0.5 g) and centrifuged at 4500 rpm for 10 min at 4 °C. The optical density of the supernatant was recorded at 470, 663, 645, and 665 nm (Porra, 2002).

The chlorophyll content was estimated by the following formula:

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

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

Car (mg/ml) = (1000 A₄₇₀–2.13 Chl a–97.64 Chl b)/209

Assay of H2O²

The H₂O₂ content was measured through the protocol of Noreen and Ashraf (2009). Fresh leaves and roots were dissolved with 0.1 % trichloroacetic acid (TCA), followed by supernatant collection through centrifugation at 12000 rpm for 15 min. The reaction mixture containing 0.5 ml of 10 mM phosphate buffer (pH-7.0), 0.5 ml of the supernatant, and 1ml of 1 M KI in a cuvette was measured at a 390 nm wavelength to estimate the content. It was calculated by using the molar extinction co-efficient $0.28 \mu M^{-1}$ cm⁻¹ and denoted as μ M g^{-1} f.w.

Assay of carbohydrate content

The reducing sugar (RS) content was determined utilizing the protocol of Afzal et al (2008). In a similar manner, the ethanol extract (3 ml) of leaves and roots were added to 3 ml of 3, 5-dinitro-salicylic acid (DNSA) solution. The mixture was heated for 5 min and1 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. Further, the content of non-reducing sugar (NS) was estimated by deducting the RS from the total soluble sugar (TS) content. The TS content was measured using the protocol of Verma et al (2001). The ethanol (1ml) extract of leaves and roots tissues were 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 as mg g^{-1} f.w.

Assay of antioxidant enzymes: SOD and CAT

The SOD activity was assayed by photo-inhibition of nitro blue tetrazolium (NBT) at 560 nm using the molar extinction coefficient 12.8 L mol⁻¹cm⁻¹, by Kumari et al. (2015). The 3 ml reaction mixture containing 50 mM phosphate buffer (pH-7.8), 0.3 ml of 10 mM EDTA, 0.3ml of 130 mM methionine, 0.25 ml of distilled water, 0.3 ml of 750 μ M NBT, and 50 μ l extracted enzymes was taken in sterilized test tubes and placed under a fluorescent lamp for 10 min. The 1 unit (U) of SOD activity is considered as the quantity of enzyme required to cause 50 % inhibition of the reduction of NBT.

% of inhibition= $[1 - Absorbance of each sample / Absorbance of the control] \times 100$

The CAT activity was determined by Zhang et al. (2021). The reaction mixture containing 50 µl of 30 mM H₂O₂, and 2.9 ml of 50 mM of enzyme extract was taken in a cuvette. The decreased absorbance was estimated at 240 nm for 3 min using the molar extinction coefficient 40 mM⁻¹ cm⁻¹ and denoted in U g^{-1} f.w.

Statistical analysis

For the various parameters of control and *Fusarium* infected leaves and roots, values are shown as the 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 $p < 0.05$ (*). Data was given as mean \pm standard error of mean (SEM).

III. RESULTS

Morphological analysis of Fusarium infected seedlings

The leaf length was observed to be decreased by 1.66-fold in *Fusarium* infected seedlings as compared to control seedlings (Fig. 1A). Similarly, the root length was reduced significantly by 1.55-fold in *Fusarium* infected than control seedlings (Fig.1B). The leaves' surface area was shrunk, edges curled inward, with wilting symptoms were observed in *Fusarium* infected seedlings. In addition, the yellowish color was occurred due to chlorosis and also stunted growth was found in infected seedlings, while all these symptoms were absent in control seedlings (Fig. 1C).

Physiological analysis of Fusarium infected seedlings

A significant reduction of FW and DW were observed by 1.44 to 2.4-fold in leaves and roots of *Fusarium* infected seedlings as compared to control (Fig. 2A and B). The RWC content was also reduced by ̴1.2 to 2.0-fold in leaves and roots of *Fusarium* infected seedlings as compared to control (Fig. 2C).

Estimation of DR and oxidants in Fusarium infected seedlings

The DR was increased by ¹0.0-fold in leaves and roots of *Fusarium* infected seedlings as compared to control (Fig. 3A). In addition, the DR of roots was higher than leaves of infected seedlings. The H_2O_2 content, an indicator of oxidative damage due to imbalance of redox system, was increased by ~2.6 and 3.7-fold in leaves and roots of *Fusarium* infected seedlings as compared to control, respectively (Fig. 3B). The H2O2 content was observed to be higher in roots than leaves of *Fusarium* infected seedlings.

Photosynthetic analysis of Fusarium infected seedlings

Chlorophyll (Chl) is a photosynthetic component that is utilized in the light reaction and the Calvin cycle. It converts inorganic compounds into organic compounds. The Chl a was decreased by 2.3-fold in leaves of *Fusarium* infected seedlings as compared to control leaves, whereas the Chl b decreased by ̴1.6-fold in leaves of *Fusarium* infected seedlings as compared to control. Moreover, the Car content decreased 3.4-fold in leaves of *Fusarium* infected seedlings as compared to control (Fig. 4A). 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, osmoprotectants, and also involved in different metabolic pathways. The RS content was increased by 7.2-fold in leaves of *Fusarium* infected seedlings as compared to control, whereas NS was decreased in leaves of *Fusarium* infected seedlings by 3.9-fold than control leaves. The TS content was decreased in leaves of *Fusarium* infected seedlings by 1.71-fold than control (Fig. 4B). In addition, roots of *Fusarium* infected seedlings showed an increased RS content of~3.5-fold than control, whereas NS was reduced in roots of *Fusarium* infected seedlings by 1.25-fold. The TS content was reduced in roots of *Fusarium* infected seedlings by 1.64-fold than control (Fig. 4C).

Estimation of antioxidant enzymes activity in Fusarium infected seedlings

The effect of *Fusarium* infection on the activity of antioxidant enzymes like SOD, and CAT were measured in leaves and root tissues for quenching ROS. The SOD activity was enhanced by ̴1.9-fold in leaves of *Fusarium* infected seedlings than control. The SOD activity was increased also by 0.4-fold in roots of *Fusarium* infected seedlings than control (Fig. 5A). Similarly, the CAT activity was increased by 1.3- fold in leaves of *Fusarium* infected seedlings than control. The root tissues also showed an enhanced CAT activity by ~0.4-foldin *Fusarium* infected seedlings than control (Fig. 5B).

IV. DISCUSSION

Wheat, a commercial significant agricultural plant, has previously been shown to acquire susceptibility to a variety of distinct fungal diseases, with *F. oxysporum* one of the most prominent plant pathogenic species responsible for massive crop losses. For the formulation of effective ways to manage wheat susceptibility, it is necessary to explore the physiological and biochemical responses during plant infection.

In the present study, the reduced leaf and root length was observed in *Fusarium* infected seedlings, which indicating the interruption of physiological activities at cellular level. It is already reported that, *Fusarium* infection can reduce the growth and development of plants through influencing metabolic pathways and retards plant growth in various studies (Subba and Mathur, 2022). Phenotypically, the *Fusarium* infected seedlings exhibited more yellowing and wilting than control seedlings (Chhabra et al. 2020).

Plant growth is regulated by the physiological functions. The reduced shoot and root development, and physiological abnormalities (photosynthesis pigment degradation and electron flow impairment) lead to a loss of biomass deposition (Fahad *et al.*2017). In our investigation, we noticed that when seedlings are subjected to *Fusarium* inoculation, their biomass decreases. Because *Fusarium* infection promotes ABA-mediated stomata closure, which disrupts the Calvin cycle's normal electron flow for carbon breakdown. Therefore, the fresh and dry matter generation was inhibited and also reduced the rate of $CO₂$ assimilation (Micol-Ponce et al. 2015). Moreover, the infection damaged the vascular system, and cortical tissues, which induced the loss of carbohydrates and promotes osmotic stress in roots of *Fusarium* infected seedlings. Gradually, the osmotic stress exosmosis the water content from tissues, and ultimately, reduced RWC content inside the plants (Guenther and Trail, 2005).Sometimes, the high numbers of *Fusarium* mycelia adherence can damaged vascular bundles of root more rapidly than leaves and as a result, unable to absorb water (Ahluwalia et al. 2021). The DR was also enhanced in *Fusarium* infected seedlings which indicating the severity of pathogenesis in wheat plants (Naz et al. 2021).The pathogenesis is occurred due to the imbalance of homeostasis between oxidants and antioxidant enzymes at cellular system. It has been reported that under *Fusarium* infection, the H₂O₂ is produced rapidly and accumulated, which is extremely dangerous to macromolecules and causes pigments destruction, carbohydrates oxidation and also causes disease progression in leaves and root tissues of *Fusarium* infected seedlings (Bi et al. 2021).

Plant propagation is regulated by photosynthetic pigments and photosynthesis rate (Tang et al. 2015). The photosynthetic pigments like Chl a, Chl b, and Car content was reduced in *Fusarium* infected seedlings, similarly, reported in *Maluspumila* Mill. leaves, and also in phytoplasma infected apple fruits (Bertamini et al. 2003). The pigments were declined due to photosystems complex and chloroplast membranes interacted with H2O2, which leads to progressive chlorosis, dysfunction of PSI and PSII in chloroplasts, and leads to cell death. Further, it also reduces the sugar contents in the leaves and roots of coconut palms, maize, and papaya leaves for inhibiting the Calvin cycle (Ambastha et al. 2015).The carbohydrates (primary metabolites) that served as the progenitor of secondary metabolites, which maintains the physiological functions at the cellular level (Mandal et al. 2022). The RS like glucose and fructose was abundant in both leaves and roots of *Fusarium* infected seedlings due to breakdown of NS sucrose by the activation of activity of sucrase enzyme. The RS contents were high during infection in bean cv. Widusa (De Ron et al*.* 2022). In contrast, the NS was low in leaves and rootsof *Fusarium* infected seedlings indicating the more hydrolysis of sucrose than control. In addition, the TS were also reduced in *Fusarium* infected seedlings than control. Similarly, the TS were also reduced in *Theobroma cacao* plants infected with *Crinipelis perniciosa,* and also in sunflower (*Helianthus annuus* L.) infected with sunflower chlorotic mottle virus (Arias et al. 2003). These carbohydrates are crucial for osmotic adjustment which was less in infected seedlings, indicating the low RWC and biomass (Farooq et al*.* 2017), and finally, reduced the growth and development.

SOD and CAT, play a part in eliminating ROS and regulating the cellular redox balance. Many workers have been studied that antioxidant enzymes SOD, and CAT activity increased during pathogenesis in plants. In combating H_2O_2 , the SOD and CAT activity was increased in leaves and roots of wheat seedlings to maintain the cellular redox state against *Fusarium* infection. A similar result was shown in tomato plants during *B. cinerea* infection (Zheng et al. 2015). The enhanced antioxidant enzymes activity was indicated the defense response in wheat seedlings during *Fusarium* infection.

V. CONCLUSION

In conclusion, our results revealed that *F. oxysporum* infection induces the physio-biochemical response in wheat seedlings. This study can serve as an assessing tool in wheat defense and/or susceptible response towards different biotic stress factors.

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Figures

Fig. 1. Phenotype of wheat seedlings : A. Growth and development of seedlings, B. Leaf length, C. Root length. * denote significance at $p<0.05$ with SEM.

Fig. 2. Physiological changes in *Fusarium* infected seedlings : A. Fresh weight, B. Dry weight, and C. RWC. * denote significance at p<0.05 with SEM.

Fig. 3. Disease rating and oxidant content in *Fusarium* infected seedlings :A. DR(%), and B. H_2O_2 content. * denote significance at p<0.05 with SEM.

Fig. 4. Biochemical changes in *Fusarium* infected seedlings:A. Pigment contents, B. Sugar contents in leaves, and C. Sugar contents in roots. * denote significance at p<0.05 with SEM.

Fig. 5. Antioxidant enzymes activity in Fusarium infected seedlings : A. SOD activity, and B. CAT activity. * denote significance at p<0.05 with SEM.