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Screening of Defense Response against *Exserohilum Turcicum* (Pass.) Leo. and Suggs. Causing Turcicum Leaf Blight (TLB) in Maize (*Zea mays*)

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ABSTRACT
Host plant resistance is the major method used to control Turcicum Leaf Blight and usually results in a distinct reduction in fungal colonization. Such resistance is associated with biochemical, molecular and morphological changes around the infected site of host tissues. In general, resistance in plants against different diseases has been correlated with various biochemical substances. The research mainly focuses on isolation, purification, identification and maintain of E. turcicum and screening of defense response against E. turcicum causing Turcicum Leaf Blight (TLB) in maize. The maize plants of varieties Jaunpuri, DMRH-1301 and DMRH-1308 were carefully inoculated with spore suspension of E. turcicum for creating artificial epiphytotic conditions in the pots following swab/ whorl drop method of inoculation and all biochemical observations [Ascorbate Peroxidase (APOX), Guaiacol Peroxidase (GPOX), Catalase (CAT), Superoxide Dismutase (SOD), Polyphenol Oxidase (PPO), Phenol (PHL), Total Protein Content (PRT), Chlorophyll a (CHLa), Chlorophyll b (CHLb) and Total Chlorophyll Content (TCC)] were studied in three varieties categorised as Jaunpuri-Untreated, Jaunpuri-Treated (susceptible), DMRH-1301 and DMRH-1308 (resistant) of maize at four stages: (i) 0 HAI (Hours after Inoculation), (ii) 24 HAI, (iii) 48 HAI and (iv) 72 HAI for biochemical assay. For biochemical estimation, results revealed that all enzymes and metabolites (APOX, GPOX, CAT, PPO, PHL, PRT, CHLa, CHLb and TCC) used in the present study except SOD showed increase in activity/ content in un-inoculated susceptible and resistant variety while APOX, GPOX, CAT and PHL activity increase up to 48 HAI and slow decrease at 72 HAI was observed in inoculated susceptible variety and vice-versa in SOD. Maximum amount of total chlorophyll, protein and PPO was noticed in the leaf of un-inoculated susceptible and resistant plants of maize but low content was noticed in inoculated susceptible variety.
Key Words: *Biochemical assay, Exserohilum turcicum, Maize, Reactive Oxygen Species (ROS), Turcicum Leaf Blight*

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INTRODUCTION

Maize (*Zea mays* L., 2n=20), also known as corn is the principal cereal worldwide, originated in Mexico and Central America but because of its large adaptability and high productivity capacity, it is grown over a broad range of locations around the world. Maize belongs to the clade monocots, tribe Maydeae and the grass family, Poaceae. With a notable productive capacity among the cereals after wheat and rice, maize is the third most significant grain crop. Maize is cultivated over an area of 191.05 million hectares with an annual production of about 1,120.47 million tonnes and average productivity of 5.86 tonnes per hectare at global level. Maize cultivation area in India, according to the report was 9.20 million hectares with an annual production of about 27.80 million tonnes and average productivity of 3.02 tonnes per hectare [32].

Among the biotic stresses affecting maize, Turcicum Leaf Blight (TLB) also called as Northern Corn Leaf Blight (NCLB) caused by *Exserohilum turcicum* (Pass.)Leo. and Suggs. is a ubiquitous foliar disease having worldwide significance. First report of TLB was from the USA, New Jersey in 1878. Severe outbreak of TLB was reported in Connecticut in 1889 [11]. Favorable environmental conditions of TLB are elevated

humidity and mild temperature. TLB in India, the disease is prevalent in H.P, U.P., A.P., Orissa Karnataka and Uttarakhand.

Plants have very complex mechanism(s) of regulating defense reactions and so, a detailed investigation of pathogen and host tissues may prove to be very successful. Fungicides can minimise progress of disease but are not cost-effective and cause ecological pollution. The most suitable strategy to manage diseases is the development of resistant varieties. The ability of a host plant to defend itself against a pathogen is administered by the environmental conditions and genetic constituents. Host plant resistance is the most important strategy to manage TLB and causes discrete fungal colonization reduction. Such resistance is coupled with biochemical, molecular and morphological changes near infected site of host tissues. Resistance against several different diseases in plants has been linked to several biochemical constituents. Relationship linking biochemical factors in maize with resistance to TLB is very scarce. Thus, present research focuses on identification of biochemical factors linked to various grades of TLB resistance [27].

For studying defense response against *Exserohilum turcicum*, the role of antioxidant enzymes and oxidative burst representing that ROS play very significant role as signalling molecule in maize varieties (*Jaunpuri*, DMRH-1301 and DMRH-1308). Various antioxidant enzymes and metabolites activity changes, such as Ascorbate Peroxidase (APOX), Guaiacol Peroxidase (GPOX), Catalase (CAT), Superoxide Dismutase (SOD), Polyphenol Oxidase (PPO), Phenol (PHL), Total Protein Content (PRT), Chlorophyll a (CHLa), Chlorophyll b (CHLb) and Total Chlorophyll Content (TCC) during artificial inoculation of TLB pathogen in maize.

Changes in enzyme activities involved in the ROS pathway could also be involved in the increased production of active oxygen species. This observed increase in active oxygen species showed that changes in the redox processes at the plasma membrane may be concerned with the plant defense response against pathogens [33].

MATERIALS AND METHODS

Biochemical assay

The maize seeds (cv. *Jaunpuri,DMRH1301 and DMRH 1308)* were surface sterilized using 0.1% sodium hypochlorite solution for one minute and then rinsed thrice in sterilized distilled water. The treated seeds were dried under shed. Soil were collected from the field area and mixed uniformly with FYM@200g/kg of soil. Soil sterilization was done with formalin (40%) of 5 ml formalin diluted with 20 ml of water for 4 kg of soil. From 3 categories of each maize variety 32 (32= 8×4R) seeds, *Jaunpuri* seeds (susceptible to TLB disease) were sown in 4 replications (4 seeds/pot i.e., 16 seeds) and DMRH-1301 and DMRH-1308 seeds (resistant to TLB disease) were sown in 2 replications each (4 seeds/pot, i.e. 8 seeds of DMRH-1301 and 8 seeds of DMRH-1308) in Earthen pots filled with already prepared soils.

Inoculation of pathogen in plants

Fifteen days old pure culture of *E. turcicum* multiplied on PDA media was used for artificial inoculation of genotypes. The spore suspension prepared in sterilized distilled water having spore load of 5 x 10^4 spores per ml (or 35-40 spores at 10X microscopic field) was used. After sowing for 35 days, the maize plants of varieties *Jaunpuri*, DMRH-1301 and DMRH-1308 (2 each) were carefully inoculated with spore suspension of pathogen and fully colonized sorghum grain culture for creating artificial epiphytotic conditions in the field following swab and whorl drop method of inoculation. The inoculated plants were misted with water after 24 hours of inoculation to create optimum humidity for disease expression. Un-inoculated checks were also maintained. Regular monitoring was done and all biochemical observations were studied in three varieties categorised as *Jaunpuri*-Untreated, *Jaunpuri*-Treated (susceptible), DMRH-1301 and DMRH-1308(resistant) of maize (2 replication each) at four stages: (i) 0 hours after inoculation (0 HAI), (ii) 24 HAI, (iii) 48 HAI and (iv) 72 HAIand 3 replicates were taken for sampling after each stage for biochemical assay.

Observations were recorded 20 days after inoculation for the development of symptoms. The plants showing typical blight symptoms were collected, the pathogen from these infected hosts were re-isolated on PDA and observed under microscope for morphological characters.

Biochemical analysis

Fresh first lower leaf samples were collected and washed twice with deionised water and all biochemical observations were studied in three varieties categorised as *Jaunpuri*-Untreated, *Jaunpuri*-Treated (susceptible), DMRH-1301 and DMRH-1308 (resistant) of maize (2 replication each) at four stages: (i) 0 HAI, (ii) 24 HAI, (iii) 48 HAI and (iv) 72 HAI and 3 replicates were taken for sampling after each stage for biochemical assay.

Determination of enzymes
Reactive oxygen species (ROS) related enzymes
Ascorbate peroxidase (APOX), guaiacol peroxidase (GPOX), catalase (CAT) and superoxide dismutase (SOD) were extracted by homogenizing one gram of in vitro grown 25-30 days old leaves in 10 ml of the extraction buffer composed of 50 mm Potassium Phosphate buffer (pH 7.0), 10 g l-1 PVP, 0.2 mm EDTA and 10 mm triton x-100 using a mortar and pestle. The resulting homogenate was centrifuged at 12,000 x g for 20 min at 4ºC and the supernatant was used for the determination of APOX, GPOX, CAT and SOD activities. The procedure employed for extraction of Polyphenol Oxidase (PPO) enzymes was similar to the one used above except that the extraction buffer was 0.1x Phosphate buffer (pH 6.8) [7].
***Ascorbate peroxidase* (APOX):**
APOX (EC 1.11.1.11) activity was determined immediately in fresh extract and was assayed. 3 ml reaction mixture containing 50 mM sodium phosphate buffer pH 7.0, 0.1 mM H_2O_2, 0.5 mM ascorbic acid, 0.1 mM EDTA and 0.1 ml enzyme extract. The hydrogen peroxide dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm (ε = 2.8 mM^{-1} cm^{-1}), [24].
Guaiacol peroxidase (GPOX):
GPOX (EC 1.11.1.7) activity was measured in the homogenates by measuring the increase in absorption at 470 nm due to the formation of tetraguaiacol (ε = 26.6 mM^{-1} cm^{-1}) in a reaction mixture containing 50 mM sodium phosphate buffer pH 7.0, 0.1 mM EDTA, 0.05 ml enzyme extract, 10 mM guaiacol and 10 mM H_2O_2 [7].
Catalase (CAT):
CAT (EC 1.11.1.6) activity was determined in the homogenates by measuring the decrease in absorption at 240 nm as H_2O_2 (ε = 39.4 mM^{-1} cm^{-1}) was consumed according the method of Aebi [1] and enzyme activity expressed as µmol H_2O_2 oxidized min^{-1} g^{-1} protein. The 3 ml mixture containing 50 mM sodium phosphate buffer (pH 7.0), 10mM H_2O_2 and 50 µl enzyme extract.
Enzyme unit of APOX, GPOX and CAT was calculated as:

$$\mathbf{mM/min/gprotein} = \frac{\mathbf{\Delta O.D.}}{\mathbf{Enzymeconc.(g) \times mg/gproteinx\varepsilon}}$$

Where, ε = Extinction coefficient
Enzyme conc. (g) = Amount of enzyme in 3 ml reaction mixture
Superoxide dismutase (SOD):
SOD activity was determined by measuring the inhibition in photoreduction of Nitroblue Tetrazolium (NBT) by SOD enzyme. The reaction mixture contained 50 mM Sodium Phosphate buffer (pH 7.6), 0.1 mM EDTA, 50 mM sodium carbonate, 12 mM l-methionine, 50 µM NBT, 10 µM riboflavin and 100 µl of crude extract in a final volume of 3.0 ml. A control reaction was performed without crude extract. The sod reaction was carried out by exposing the reaction mixture to white light for 15 min at room temperature. After 15 min incubation, absorbance was recorded at 560 nm using a spectrophotometer. One unit (U) of SOD activity was defined as the amount of enzyme causing 50% inhibition of photochemical reduction of NBT [4].

$$\boldsymbol{SODUnit} = \frac{\boldsymbol{O.D.control\ (withoutenzyme)}}{\boldsymbol{O.D.sample}} - \mathbf{1} \times \frac{\mathbf{1}}{\boldsymbol{enzymeconc.\ (g)}}$$

$$\mathbf{SODUnit/gprotein} = \frac{\boldsymbol{SODUnit}}{\boldsymbol{mg/gprotein}}$$

Phenyl propenoid pathway related enzymes and total phenol estimation
Polyphenol oxidase (PPO) assay (EC 1.14.18.1):
Enzyme extraction of separated Leaf tissue of 100 mg was homogenized in 1 ml of 0.1 M sodium phosphate buffer, pH 6.8. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was used for enzyme assay. Enzyme assay: The reaction mixture contained 2.9 ml of 50 mM of catechol prepared in 0.1 M sodium phosphate buffer, pH 6.8. The reaction was initiated by the addition of 100 µl of enzyme extract. The change in the colour due to the oxidized catechol was measured at 280 nm for 1 minute at an interval of 15 seconds. The enzyme activity was expressed as unit min^{-1} g^{-1} protein. One PPO unit is defined as the change in absorbance per minute under the above assay conditions [31].

$$\mathbf{Unit/mg} = \frac{\mathbf{\Delta A280/min \times 1000}}{\mathbf{mgenzymeinreaction}}$$

Total phenol estimation (PHL):
Phenol content was estimated by Malick and Singh [19]. Leaf tissue (100 mg) was extracted with 5 ml of 80% ethanol and centrifuged at 3000 rpm for 10 minutes. Extraction was repeated 4 times with 80% ethanol and supernatants were collected into 25 ml volumetric flasks. Final volume of the extract was prepared to 25 ml with 80% ethanol. One ml of supernatant was taken from ethanol extract and

evaporated to dryness in water bath. One ml of millipore water in each test tube and 0.5 ml of Folin and Ciocalteu reagent (1:1 with water) was added and kept for 3 min. After this 2 ml of 20% Na_2CO_3 was added and thoroughly mixed. The tubes were placed in boiling water for exactly one minute and cooled in ice water.

The absorbance was measured at 650 nm against a reagent blank. A standard graph was prepared using pyrocatachol ranging between 0-25 µg concentrations. The standard curve was obtained by plotting the absorbance at 650 nm against µg of reaction mixture standard samples. As expected, we obtained a linear graph. The equation of the line was calculated using Microsoft Excel software. With this equation, the amount of phenol in the unknown samples was calculated.

The amount of phenols present in the sample was calculated as:

$$\boldsymbol{Phenol\ (mg/g)\ =\ SampleO.D.\ \times StandardO.D.\ \times Dilutionfactor}$$

Total Protein Determination (PRT):

Concentration of protein of each enzyme extract was estimated by Lowry method [16]. To 1.0 ml of the above protein solution was added 5 ml of freshly prepared alkaline copper sulfate reagent (mixture of 2 ml of (10 ml of 1.56% Copper Sulphate solution was mixed with 10 ml of 2.37% Sodium Potassium Tartarate solution) with 100 ml of (50 ml of 2% Sodium Carbonate was mixed with 50 ml of 0.1 N NaOH) and properly mixed. A blank was also set up which contained 1 ml of 0.1N NaOH in place of protein sample. After keeping for 10 min at room temperature, 0.5 ml of 1N Folin-Ciocalteau Reagent was added and left for 30 min in dark. The absorbance of the resulting blue color was recorded at 660 nm against the blank. The amount of soluble proteins was calculated in mg g^{-1} fw with the help of standard plot of BSA (0-150 µg). The standard curve was obtained by plotting the absorbance at 660 nm against µg of protein in BSA standard samples. As expected, we obtained a linear graph. The equation of the line was calculated using Microsoft Excel software. With this equation, we calculated the amount of protein in the unknown samples.

Chlorophyll content (CHLa, CHLb and TCC):

Chlorophyll was estimated in leaves of control and all the treatments following the method of Coombs *et al*. [6]. *In vitro* grown one gram leaves of each treatment were homogenized in 10 ml of 80% acetone (v/v) in chilled mortar and pestle followed by centrifugation at 3,000 x g for 10 min. The supernatant from each treatment was collected in separate test tubes and the extraction was repeated with 80% acetone. The final volume in each tube was made up to 10 ml by adding 80% acetone and its absorbance was read at 647 and 664 nm against 80% aqueous solution of acetone. The chlorophyll content was calculated as follows:

$$\boldsymbol{Chlorophylla = 13.19A664 - 2.57A647}$$

$$\boldsymbol{Chlorophyllb = 22.10A647 - 5.26A664}$$

$$\boldsymbol{TotalChlorophyllContent\ = 7.93A664\ +\ 19.53A647}$$

Where, A_{647} and A_{664} represent the absorbance at 647 nm and 664 nm, respectively. The numbers 2.57, 5.26, 7.93, 13.19, 19.53 and 22.10 are absorption coefficients. The levels of chlorophyll were converted to mg g^{-1}FW.

All the data was analysed using ANOVA (Analysis of Variance) in MS Office Excel 2007 Sheet within the framework of t-test. The means were separated using S.Em. (Standard Error of the mean), C.D. (Critical Difference) and C.V. (Coefficient of Variation) to determine whether there were significant differences among the treatments in comparison to control.

RESULTS AND DISCUSSION

A lot of transformations in essential biochemical processes like enzyme and metabolite changes in the host plant occur during biotic stress i.e., pathogenic infection. Large fluctuations are observed in biochemical constituents like antioxidant enzymes and metabolites such as phenol, protein, chlorophyll, etc.

Biochemical estimation

After sowing for 35 days, the maize plants of varieties *Jaunpuri*, DMRH-1301 and DMRH-1308 were carefully inoculated with spore suspension of *E. turcicum* and fully colonized sorghum grain culture for creating artificial epiphytotic conditions in the pots following whorl drop method of inoculation. All biochemical observations were studied in three varieties categorised as *Jaunpuri*-Untreated, *Jaunpuri*-Treated (susceptible), DMRH-1301 and DMRH-1308(resistant) of maize (2 replication each) at four stages: (i) 0 hours after inoculation (0 HAI), (ii) 24 HAI, (iii) 48 HAI and (iv) 72 HAI (**Plate-3**) and 3 replicates were taken for sampling after each stage for biochemical assay.

For biochemical estimation, the enzymes related to reactive oxygen species (ROS) generation and metabolism such as Ascorbate Peroxidase (APOX), Guaiacol Peroxidase (GPOX), Catalase (CAT) and

Superoxide dismutase (SOD) activity were analyzed and enzyme related to metabolism of phenol such as Polyphenol Oxidase (PPO) activity was also recorded along with total phenol estimation (PHL). Total protein (PRT), chlorophyll a (CHLa), chlorophyll b (CHLb) and total chlorophyll content (TCC) were also observed. However, information on correlation between resistance to TLB and biochemical parameters in maize is very limited.

Reactive oxygen species (ROS) related enzymes

Ascorbate peroxidase (APOX):

In the present research, increase in APOX activity was observed at time interval 0-72 HAI (22.017-26.613 µM/min/g protein) in *Jaunpuri*-Untreated i.e., Control. But in *Jaunpuri*-Treated, i.e. after inoculation, APOX activity increased at 0-48 HAI (29.420-39.525 µM/min/g protein) and suddenly decreased at 72 HAI (24.140 µM/min/g protein) as compared to *Jaunpuri*-Untreated and hybrid DMRH-1301 and DMRH-1308 plants (**Fig.-1**).

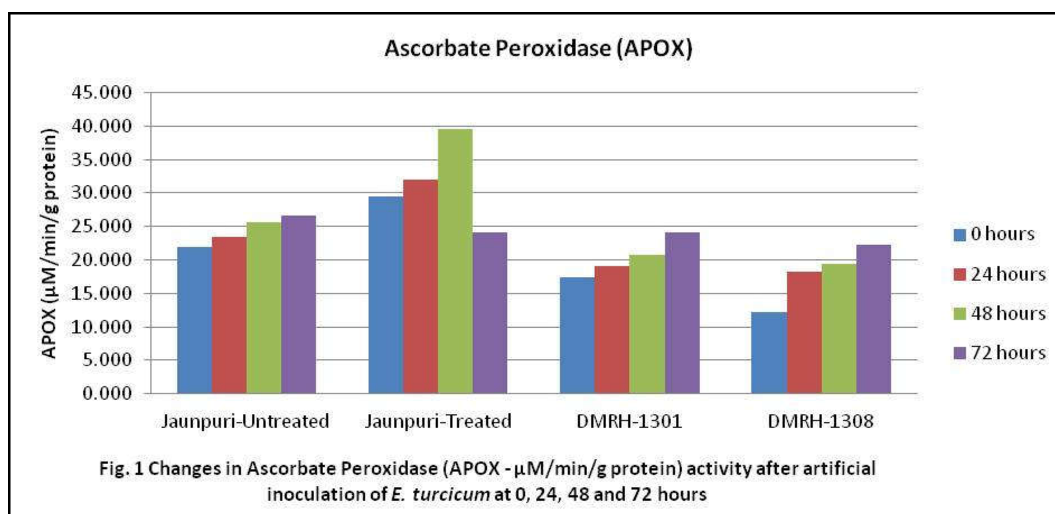

Fig. 1 Changes in Ascorbate Peroxidase (APOX - µM/min/g protein) activity after artificial inoculation of *E. turcicum* at 0, 24, 48 and 72 hours

The cytosolic APOX isoenzyme has been considered one of the most important enzymes in defense against H_2O_2. It is the first enzyme to receive the signals produced during stress, acting very quickly to prevent severe damage to cell and/or whole tissue because of its cellular localization. APOX activity has increased in plants in response to various stress conditions, such as fungi infection, drought, water lodging, heavy metals and salinity [21].

Guaiacol peroxidase (GPOX):

In the present research, increase in GPOX activity was observed at time interval 0-72 HAI (0.574-0.638 µM/min/g protein) in *Jaunpuri*-Untreated i.e., Control. But in *Jaunpuri*-Treated, i.e. after inoculation, GPOX activity increased at 0-48 HAI (1.110-1.176 µM/min/g protein) and suddenly decreased at 72 HAI (1.091 µM/min/g protein) as compared to *Jaunpuri*-Untreated and hybrid DMRH-1301 and DMRH-1308 plants (**Fig.-2**).

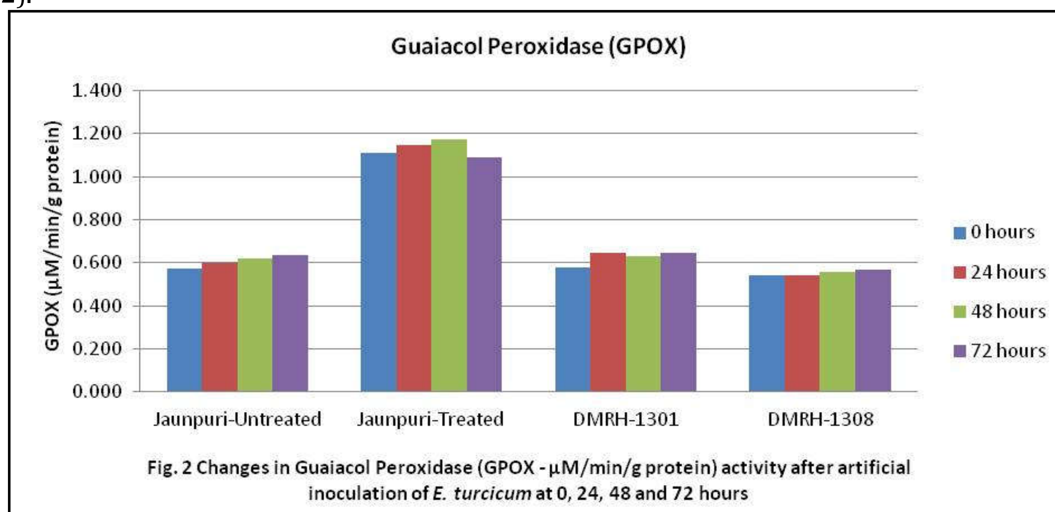

Fig. 2 Changes in Guaiacol Peroxidase (GPOX - µM/min/g protein) activity after artificial inoculation of *E. turcicum* at 0, 24, 48 and 72 hours

Degradation of indole-3-acetic acid and resistance to pathogens are the major functions of GPOX in plants [3, 10] reported that concentrations of GPOX enzymes improved considerably during colonization with arbuscular mycorrhizal fungi (AMF). The elevated GPOX activity under AMF colonization indicates an increased oxidative stress relief, hence a greater water deficiency resistance that indicates drought tolerance feature in plants.

Catalase (CAT):

Catalase is involved in the removal of H_2O_2 by reducing H_2O_2 to $2H_2O$. CATs are mainly, but not exclusively, localized to peroxisomes. Plants have multiple CATs encoded by specific genes, which respond differentially to a variety of stresses that are known to generate ROS..

The change in CAT activity upon *E. turcicum* inoculation was measured. In *Jaunpuri*-Untreated (0.856-2.228 μM/min/g protein), DMRH-1301 (0.864-2.026 μM/min/g protein) and DMRH-1308 (0.937-2.101 μM/min/g protein), CAT activity increased during the experiment period. However, in *Jaunpuri*-Treated, i.e. after inoculation, CAT activity increased at 0-48 HAI (1.793-2.254 μM/min/g protein) and suddenly decreased at 72 HAI (1.897 μM/min/g protein) (**Fig.-3**).

It is believed that the catalase enzyme plays a vital role in resistance by oxidizing phenolic compounds to quinines that are more harmful to microorganisms. The highest activation of catalase was observed in resistant control while the enzyme was less involved in susceptible control [25].

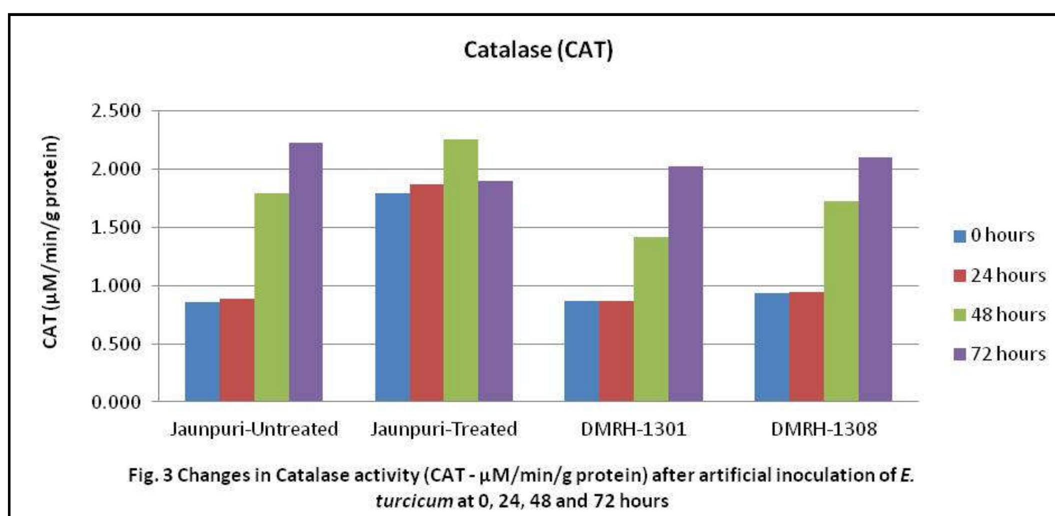

Fig. 3 Changes in Catalase activity (CAT - μM/min/g protein) after artificial inoculation of *E. turcicum* at 0, 24, 48 and 72 hours

CAT activity increased markedly in flax leaves of resistant and susceptible genotypes under powdery mildew disease [3] and *Fusarium* wilt in tomato also [12].

Superoxide Dismutase (SOD):

SOD catalyzes the reaction of dismutation of O_2^- to H_2O_2; therefore, they represent a frontline in the defense against ROS. These enzymes may be attached to a metal ion (Cu/Zn, Mn, Fe, and Ni); thus, they are categorized according to their sub-cellular site and metal co-factor. SODs are present in various organisms, such as bacteria, yeast, animals and plants. Plants have several genes encoding SODs that can be synchronized by development, tissue-specific and environmental signals [17, 18].

In this study, the SOD activity was gradually decreased at time interval 0-72 HAI in *Jaunpuri*-Untreated (0.430-0.032 Unit/g protein), DMRH-1301 (2.421-1.816 Unit/g protein)andDMRH-1308 (2.252-1.727 Unit/g protein). But in *Jaunpuri*-Treated variety i.e., after inoculation, there was decrease in SOD activity up to 48 HAI (3.476-2.557 Unit/g protein) and increase at 72 HAI (3.383 Unit/g protein) (**Fig.-4**).

Malencic *et al.* [17. 18] found that the SOD activity increased in sunflower and soybean lines with increasing oxalic acid concentration as well as inoculation with *Sclerotinia sclerotiorum*.

SOD activity was similar in both resistant and susceptible cultivars of chick pea and increased after *Fusarium oxysporum* invasion, but resistant cultivar showed earlier increasing in SOD activity [13].

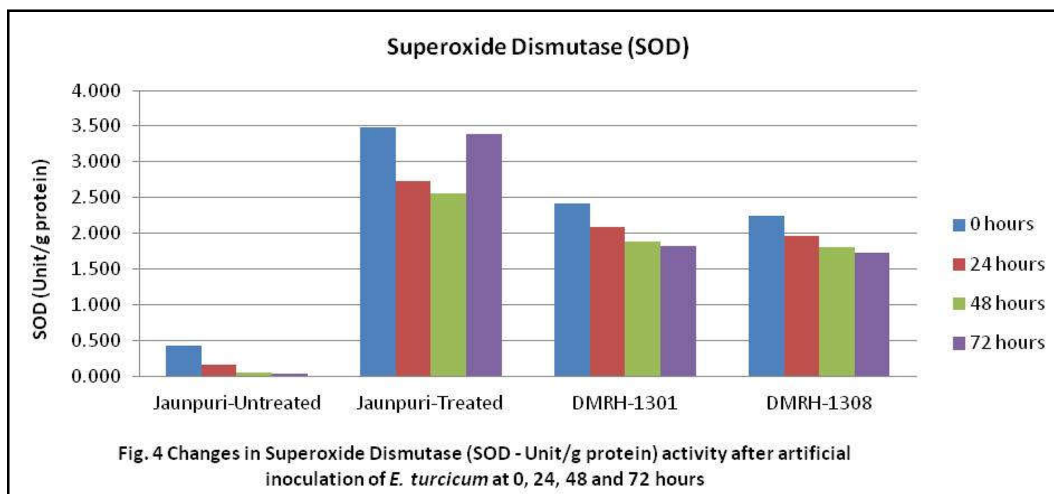

Fig. 4 Changes in Superoxide Dismutase (SOD - Unit/g protein) activity after artificial inoculation of *E. turcicum* at 0, 24, 48 and 72 hours

SOD activity increased after *Phytophthora infestans* infection in potato plants [8, 9] and in abiotic stress in various crops also (Luis *et al.*, 2018).***Phenyl propenoid pathway related enzymes and Total phenol content***

Polyphenol oxidase (PPO):

PPO activity was gradually increased at time interval 0-72 HAI in *Jaunpuri*-Untreated (Control) (1.170-5.052 Unit/mg enzyme in reaction), *Jaunpuri*-Treated (after inoculation) (1.089-5.000 Unit/mg enzyme in reaction), DMRH-1301 (0.941-1.311 Unit/mg enzyme in reaction) and DMRH-1308(resistant) (0.548-1.044 Unit/mg enzyme in reaction) plants (**Fig.-5**).

In plants, PPOs are almost ubiquitous and situated in chloroplasts and the loss of sub-cellular compartmentalization, due to senescence, wounding, interactions with pests and pathogens and handling during post-harvest processing and storage, results in contact between vacuolar phenolic substrates and PPOs [30].

PPO activity was found enhanced in mustard genotypes treated with bioagent *Trichoderma* spp., followed by *Alternaria brassicae* inoculation compared to bioassay control [26] and with the infection progress in the *A. brassicola*- Brassiceae pathosystem under optimized artificial bio-assay [25].

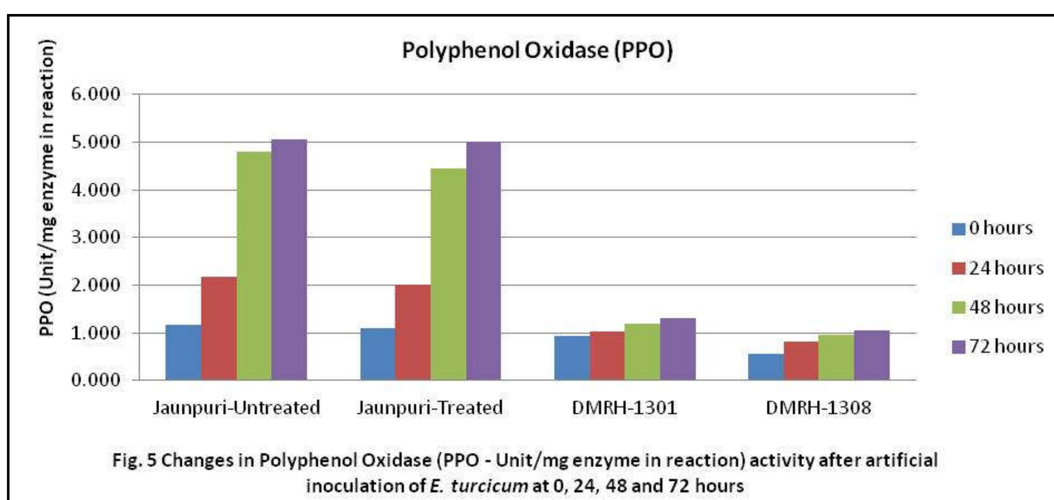

Fig. 5 Changes in Polyphenol Oxidase (PPO - Unit/mg enzyme in reaction) activity after artificial inoculation of *E. turcicum* at 0, 24, 48 and 72 hours

Total Phenol content (PHL):

Phenolic compounds are majorly found in plant tissues, like flavonoids, tannins, hydroxycinnamate esters and lignin, and possess antioxidant properties [9].Rapid accumulation of phenolic compounds at the site of infection allows the activation of antioxidants or other stress related substances and slows the pathogen growth and development. However, no single factor is sufficient to determine the resistance in plants since, it is the complex phenomenon. Dynamic role of secondary metabolites and antioxidants in expression of resistant reaction in various crops was reported [27].

In *Jaunpuri*-Untreated (17.770-51.270 mg Gallic acid/g FW), DMRH-1301 (11.520-27.353 mg Gallic acid/g FW) and DMRH-1308 (9.270-29.603 mg Gallic acid/g FW), total phenol content increased during the experiment period. But in *Jaunpuri*-Treated, i.e. after inoculation, the resulting of total phenol contents showed gradual increase at 0-48 HAI (14.437-33.270 mg Gallic acid/g FW) and decrease at 72 HAI (22.270 mg Gallic acid/g FW), as compared to control *Jaunpuri*-Untreated plant and hybrid DMRH-1301 and DMRH-1308 plants (**Fig.-6**).
Highest phenols were observed in infected leaves of resistant inbred lines compared to susceptible inbred lines. Phenol content and its enhancement during disease progress were least in susceptible varieties [15, 17, 27].

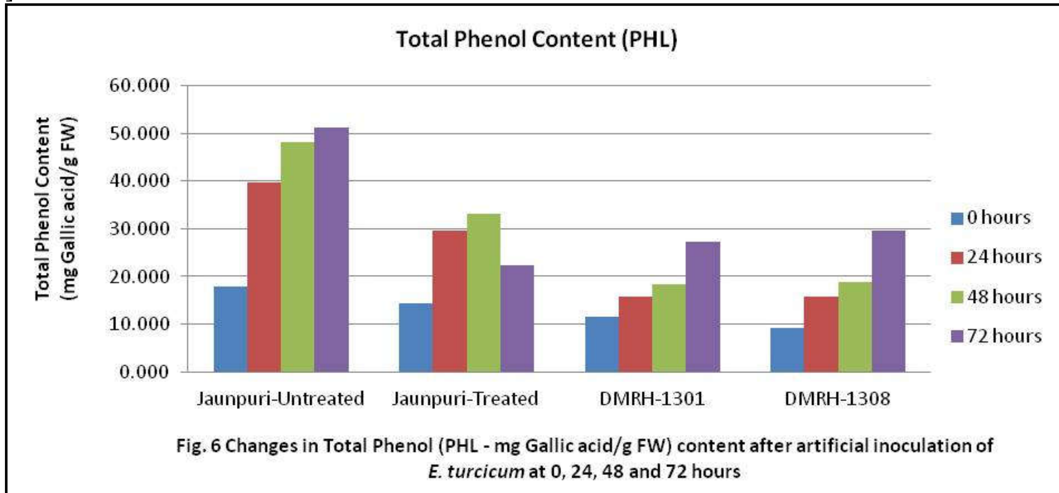

Fig. 6 Changes in Total Phenol (PHL - mg Gallic acid/g FW) content after artificial inoculation of *E. turcicum* at 0, 24, 48 and 72 hours

Rapid accumulation of phenolic compounds at the site of infection allows the activation of antioxidants or other stress related substances and slows the pathogen growth and development. However, no single factor is sufficient to determine the resistance in plants since, it is the complex phenomenon. Dynamic role of secondary metabolites and antioxidants in expression of resistant reaction in various crops was reported [28].

Total protein content (PRT):

In the present investigation, the protein content was highly reduced during 0-72 HAI in *Jaunpuri*-Treated (15.990-15.703 mg/g FW) plants compared to those of control *Jaunpuri*-Untreated (22.009-26.774 mg/g FW), DMRH-1301 (20.399-24.661 mg/g FW)andDMRH-1308 (20.494-24.554 mg/g FW) plants, where it increased during 0-72 HAI (**Fig.-7**).
Protein content was more in resistant inbred lines in both healthy and diseased ones compared to susceptible and highly susceptible inbred lines against *E. turcicum* in maize at 30, 60 and 90 DAS. The protein content decreased slightly from 30 to 90 DAS. This stage plays a crucial role for yield reduction [21].
The protein decrease may be due to degradative and denature activity. The protein biosynthesis of the host is widely thought to be considerable feature of pathogenesis, particularly during incompatible reaction.

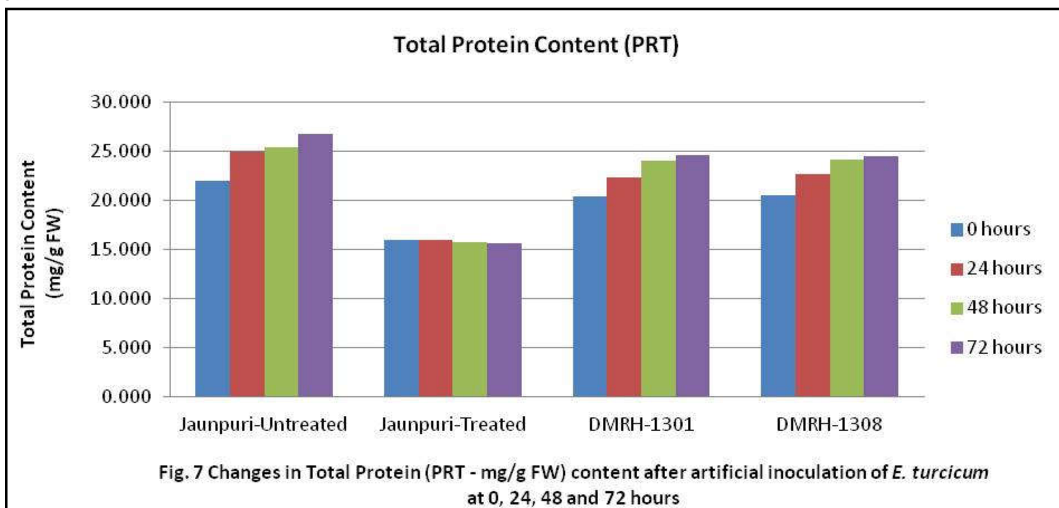

Fig. 7 Changes in Total Protein (PRT - mg/g FW) content after artificial inoculation of *E. turcicum* at 0, 24, 48 and 72 hours

Determination of Chlorophyll content (CHLa, CHLb and TCC)

In the present study, CHLa, CHLb and TCC were decreased significantly during 0-72 HAI in *Jaunpuri*-Treated (TCC: 8.981-6.567 mg/g FW) plants compared to those of control *Jaunpuri*-Untreated (TCC: 13.477-16.293 mg/g FW), DMRH-1301 (TCC: 34.388-39.189 mg/g FW) and DMRH-1308 (TCC: 29.971-36.004 mg/g FW) plants, where it increased during 0-72 HAI (Fig.-8-10).

All types of chlorophyll were more in resistant inbred lines in both healthy and diseased ones compared to susceptible and highly susceptible inbred lines against *E. turcicum* in maize at 30, 60 and 90 DAS. The chlorophyll content decreased slightly from 30 to 90 DAS. This stage plays a crucial role for yield reduction [27]. Similar results on decline of chlorophyll content after pathogen inoculation was obtained by earlier workers [2, 21]. The chlorophyll content decrease may be due to degradative and denature activity.

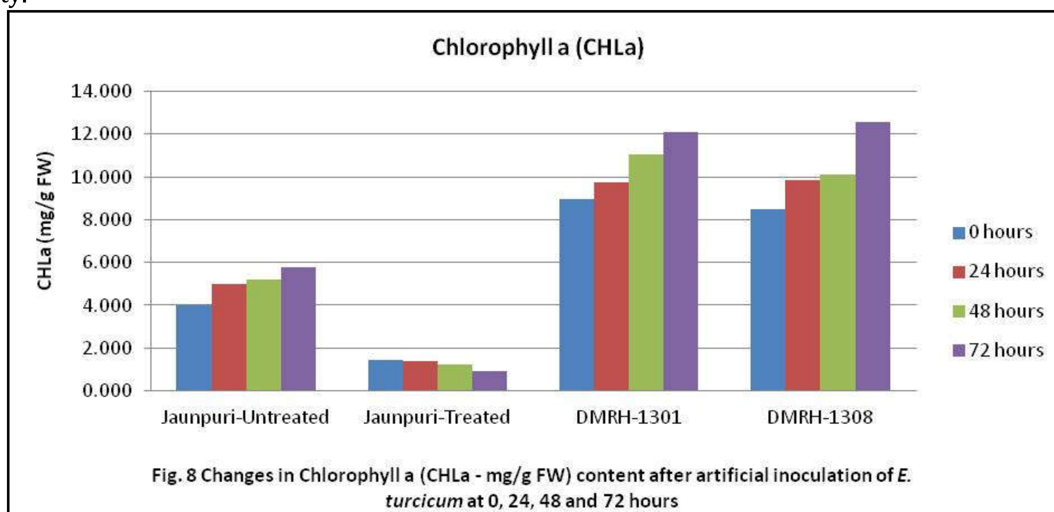

Fig. 8 Changes in Chlorophyll a (CHLa - mg/g FW) content after artificial inoculation of *E. turcicum* at 0, 24, 48 and 72 hours

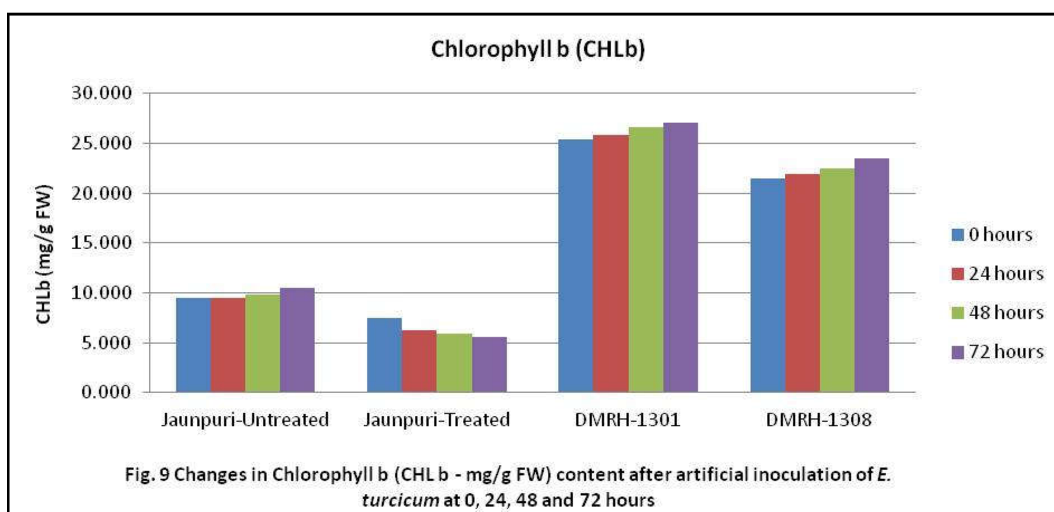

Fig. 9 Changes in Chlorophyll b (CHL b - mg/g FW) content after artificial inoculation of *E. turcicum* at 0, 24, 48 and 72 hours

APOX, GPOX, CAT, PPO, PHL, PRT, CHLa, CHLb and TCC activity were gradually increased in contrast to SOD, where activity decreased at time interval 0-72 HAI in *Jaunpuri*-Untreated and DMRH-1301and DMRH-1308 (resistant) but after inoculation with *E. turcicum,* there was an increase in APOX, GPOX, CAT and PHL activity of *Jaunpuri*-Treated variety up to 48 HAI and slow decrease at 72 HAI was observed. While there was decrease in SOD activity of *Jaunpuri*-Treated variety up to 48 HAI and increase at 72 HAI was observed. PPO activity show gradual increase in all varieties at time interval 0-72 HAI. PRT, CHLa, CHLb and TCC show gradual decrease in *Jaunpuri*-Treated plants at time interval 0-72 HAI. Results revealed that total chlorophyll and protein was observed maximum in the leaf of *Jaunpuri*-Untreated but in *Jaunpuri*-Treated with *E. turcicum*, these metabolites were observed minimum.

Based on the present study, significant differences in plant responses to oxidative stress parameters have been established between lines investigated. It has been also established that the effect of any pathogenic fungus on a host plant depends on properties of the pathogen, the host, the pathogen-host interaction and

environmental factors. These differences may point to further pathways in exploring host-pathogen relations which could ultimately lead to selection and production of new genotypes with higher levels of resistance to *E. turcicum.*

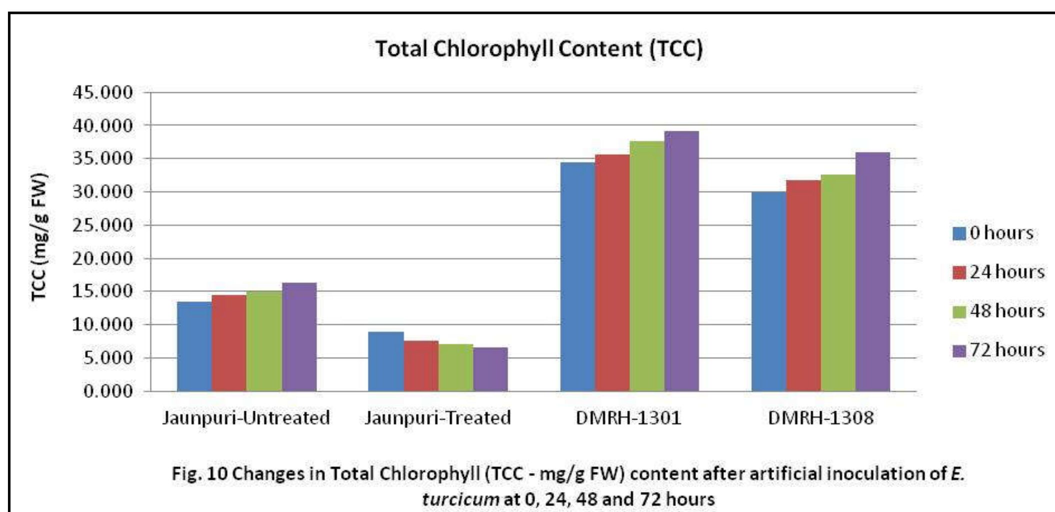

Fig. 10 Changes in Total Chlorophyll (TCC - mg/g FW) content after artificial inoculation of *E. turcicum* at 0, 24, 48 and 72 hours

CONCLUSION

Results obtained revealed that all enzymes and metabolites used in the present study except SOD showed increase in activity/ content in un-inoculated susceptible and resistant variety while APOX, GPOX, CAT and PHL activity increase up to 48 HAI and slow decrease at 72 HAI was observed in inoculated susceptible variety and vice-versa in SOD. Maximum amount of total chlorophyll, protein and PPO was noticed in the leaf of un-inoculated susceptible and resistant plants of maize but low content was noticed in inoculated susceptible variety.

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