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Oxidative stress and role of antioxidant machinery in two cultivars of sorghum (*Sorghum bicolor* L.) to combat the damage induced by *Macrophomina phaseolina*

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Abstract
The presence of *M. phaseolina* affects the sorghum cultivars and constrains the crop productivity. A pot experiment was performed using two cultivars (PJ-1430 and SU-1080) of *S. bicolor* to evaluate the difference in their response to *M. phaseolina* infection at different time intervals. The malondialdehyde quantity enhanced in the roots of sorghum plants treated with *M. phaseolina* and the damage was found to be less in PJ-1430 than SU-1080. The induction of enzymatic antioxidants (catalase, ascorbate peroxidase, superoxide dismutase, glutathione reductase) was found in roots and leaves of plants with *M. phaseolina* inoculation. Our results indicate that the cultivar PJ-1430 is found to be tolerant to *M. phaseolina* stress, associated with higher antioxidative activity and thus less oxidative damage. SU-1080 experienced maximum damage in terms of reduced antioxidant activity. Better coordination of antioxidants protected PJ-1430 from *M. phaseolina* induced damage, whereas lesser antioxidants activity in SU-1080 resulted in maximum damage.

Keywords: SOD, CAT, APX, GR, MDA, *S. bicolor*

1. Introduction

Macrophomina phaseolina, a global devastating necrotrophic fungal pathogen, infects more than 500 plant hosts [1, 2, 3].Charcoal rot of sorghum caused by *M. phaseolina*, soil and seed-borne disease of sorghum, is endemic to tropical and temperate regions of the world [4]. The disease severity may be enhanced by hot and dry environment [5] and may cause upto 100% yield losses [6]. Increased incidence of the pathogen on diverse crop species has been reported worldwide, highlighting the importance of this disease to crop production in drought prone regions [7].

Macrophomina phaseolina infects plants on almost all growth stages and are instigated by seed, soil and plant residues [8]. Plant defoliation and wilting occurs due to formation of red to brown lesions on roots and stems with production of dark mycelia and black microsclerotia [9]. It is difficult to control *M. phaseolina* due to its persistence as sclerotia in the soil and plant debris. However, low levels of productivity in sorghum are due to drought and are aggravated by charcoal rot disease which usually appears at grain maturity stage and causes severe lodging. Sorghum charcoal rot is characterized by lodging of plants as they approach maturity.

M. phaseolina induces the production of reactive oxygen species (ROS) including superoxide radical, hydrogen peroxide and hydroxyl radical which may results in membrane damage and the destruction of cellular organelles and biomolecules i.e. cause cell death due to oxidative stress such as membrane lipid peroxidation which is reflected by increased malondialdehyde (MDA) concentration, enzyme inhibition and damage to nucleic acids. To repair the *M. phaseolina* induced inhibitory effects of ROS, plants possess the antioxidative enzymes superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) and glutathione reductase [10]. Among, antioxidative enzymes, superoxide dismutase constitutes the primary step of cellular defense and dismutates $O_2^{\cdot-}$ to H_2O_2 and O_2. Further, the accumulation of H_2O_2 is restricted through the action of catalase or by ascorbate glutathione cycle, where ascorbate peroxidase is converted to H_2O. Finally, glutathione reductase catalyzes the NADPH-dependent reduction of oxidized GSSG to the reduced GSG. It has been reported that *M. phaseolina* induced damage in cereals is found to be associated with the ability of antioxidant machinery to scavenge *M. phaseolina* induced production of excessive ROS [11]. The current study was undertaken to see the role of antioxidants machinery in two cultivars of sorghum against the charcoal rot pathogen *M. phaseolina* and to examine the correlation in susceptible

and resistant cultivars and *M. phaseolina* induced oxidative damage. This study also explores the possibility of using these factors as components of resistance with phenotypic parameters for the efficient selection of charcoal rot resistant cultivars of sorghum. Selection of resistant cultivars in different species against *M. phaseolina* has proven to play a crucial role in breeding programmes.

2. Materials and methods:

2.1 Experimental design and plant growth

Sorghum seeds were obtained from Agricultural Research Institute Durgapura, Jaipur, Rajasthan. Selected healthy seeds were sterilized with 1% sodium hypochlorite solution (w/v) for 10 min, thoroughly washed with double distilled water for 1 h. For sowing, seeds were placed in pots of 15 cm diameter containing 1 kg sterilized soil and soilrite (3:1) in growth chamber under 16h/8h day/night photoperiod of 220 ± 20 $\mu E/m^2/s$ at changing day/night temperature of 24°C/20°C [12]. Primary leaves and roots of 15-day old seedlings were used for experimental purposes.

2.2 Estimation of Lipid peroxidation

Malondialdehyde (MDA) was measured by the method of [13]. The extent of lipid peroxidation was evaluated by the thiobarbituric acid reaction.

0.4 gram of frozen plant tissue (root and leaf) was homogenized in 0.1% trichloroacetic acid (1:10, w:v) and centrifuged at 10,000 rpm for 15 min. The supernatant was used for enzyme assay. One ml of the supernatant was incubated with 4 ml of 0.5% thiobarbituric acid in 20% trichloroacetic acid at 95°C for 30 min and then cooled in ice bath. After centrifugation at 10,000 g for 10 min, the absorbance of the supernatant was read at 532 nm and corrected for the non-specific absorbance recorded at 600 nm. The concentration of thiobarbituric acid reactive substances was calculated as malondialdehyde equivalent using the extinction coefficient (155 mM^{-1} cm^{-1}).

2.3 Antioxidant enzyme assays

2.3.1 Enzyme extraction

The enzyme extractions were carried out at 4°C. 0.25 gm of frozen plant tissues (roots and leaves) were ground to a fine powder in liquid nitrogen in a mortar with a pestle and extracted at a ratio 1:10 (w/v) fresh weight to extraction buffer (100 mM potassium phosphate buffer (pH 7.5) containing 1 mM EDTA, 3 mM DTT and 5% (w/v) insoluble PVPP). The homogenate was centrifuged at 10000 rpm for 30 min and the supernatant kept stored in separate aliquots at -80° C to be used for SOD, CAT, APX and GR analysis.

2.3.2 Determination of SOD

The activity of SOD was assayed by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) [14]. The enzyme extract was added to 3 ml of reaction mixture containing 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 mM NBT, 0.1 mM EDTA and 0.2 mM riboflavin. The riboflavin was added at the end and the tubes were shaken and placed 30 cm below a light source consisting of two 15 W fluorescent lamps. The reaction was started by switching on the light and was allowed to run for 15 min. The reaction was stopped by switching off the light and the tubes were covered with a black cloth. The photoreduction of NBT (production of blue formazan) was measured at 560 nm and an inhibition curve was made against various volumes of the extract. One unit of SOD was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT at 560 nm.

2.3.3 Determination of catalase activity

The activity of CAT was determined by the method of [15] with slight modification by monitoring the disappearance of H_2O_2 at 240 nm. 3 ml H_2O_2-phosphate buffer was taken in cuvette and 100 µl enzyme extract was added. The absorbance was taken in the UV-VIS-2250 Shimadzu spectrophotometer at 240 nm. The absorbance was allowed to decrease from 0.45 to 0.4 from the time required in seconds (Δt) against diluted phosphate buffer as blank. Δt should be less than 60 seconds. The activity was calculated by using extinction coefficient 0.036 $mM^{-1}cm^{-1}$. One unit of the enzyme is the amount necessary to decompose 1 mmol of H_2O_2 per min at 25°C.

2.3.4 Determination of APX activity

The activity of APX was determined according to [16]. 3 ml of 50 mM phosphate buffer (pH 7) containing 5 mM ascorbate was taken and 100µl of enzyme extract was added to it. 2.5 µl H_2O_2 was added and the reaction was determined spectrophotometrically at 290 nm. One unit of the enzyme is the amount necessary to decompose 1 µmol of ascorbate per min at 25°C. APX activity was calculated by using extinction coefficient 2.8 $mM^{-1}cm^{-1}$.

2.3.5 Determination of glutathione reductase

GR activity was assayed spectrophotometrically at 30°C as described by [17] in a reaction mixture consisting of 3 ml 100 mM potassium phosphate buffer (pH 7.5) containing 1mM 5,5"-dithiobis(2-nitrobenzoic acid), 1mM oxidized glutathione and 0.1mM NADPH. The reaction was initiated by the addition of 50 µl of plant extract. The rate reduction of oxidized glutathione was followed by monitoring the increase in absorbance at 412 nm over 2 min. GR activity was determined by monitoring the glutathione-dependent oxidation of NADPH. The activity of GR was calculated by using extinction coefficient 6.2 $mM^{-1}cm^{-1}$. One unit of enzyme was the amount necessary to decompose 1 mmol of NADPH per min at 25°C.

2.4 Statistical analysis

For statistical validity, each treatment was made in 5 replicates for estimating enzyme activity. The data were expressed as mean ± standard deviation (SD) and were analyzed by ANOVA using SPSS software. Significance of differences was checked with the Tukey's post-hoc test. Statistical significance was set at $p<0.05$.

3. Results & Discussion

3.1 Quantitative determination of MDA content in roots and leaves of *S. bicolor* infected with *M. phaseolina*

The MDA content (a measure of lipid peroxidation) in the root and leaf samples was determined in cultivar PJ-1430 and SU-1080 in *S. bicolor* plants inoculated with *M. phaseolina* and expressed in terms of MDA content µmol.gfw^{-1}. The effect of *M. phaseolina* on MDA content in root and leaf samples is presented in (Fig. 1-2).

The MDA content increased in both root and leaves in time dependent manner. An exposure of *M. phaseolina* in soil in 15 day-old *S. bicolor* plants, resulted in increase in MDA content, in PJ-1430 the increase in MDA content in root is

0.60 µmol gfw^{-1} to 3.10 µmol gfw^{-1} and 0.45 to 3.46 µmol gfw^{-1} in SU-1080. In root samples (Fig. 1) infection by the pathogen resulted in the transient increase in the MDA content in both the cultivars compared with the control; however such an increase was higher which was 6.7 fold in SU-1080 compared to cultivar PJ-1430 which was 4 fold.

ROS (H_2O_2 and $O_2^{\bullet-}$) activity frequently cause membrane damage through peroxidation of fatty acids, which may be coincided with the activity of lipoxygenase in plants as a consequence of infection. Levels of MDA gradually increased in leaves of infected sorghum plants with increasing the time of infection. When plants are subjected to pathogen attack, the equilibrium between productions and scavenging of ROS is broken, resulting in oxidative damage of proteins, DNA and lipids. MDA is the marker for lipid peroxidation released from cellular membranes of tissues and are formed by the reaction of ROS (H_2O_2 or/and $O_2^{\bullet-}$) with lipid molecules [18]. Thus, lipid peroxidation in sesamum plants has been proved to be induced by pathogens, and the subsequent products have been shown to possess antimicrobial properties and signalling function [19].

An exposure of *M. phaseolina* in soil in 15 day-old *S. bicolor* plants, resulted in increase in MDA content, in PJ-1430 the increase in MDA content in leaf was 0.54 µmol gfw^{-1} to 1.41µmol gfw^{-1} which was 2.3 folds higher than control plants and 0.41 to 1.82 µmol gfw^{-1} which was 4.4 folds higher in SU-1080 at 168 h (Fig. 2). Similarly, an increase in the MDA content in leaves of *Citrus aurantifollia* inoculated with *Xanthomonas* citr subsp. Citri [20].

3.2 Catalase activity in leaves and root of *S. bicolor* infected with *M. phaseolina*

The catalase activity of 15 days old plants was analysed at 0, 2, 4, 24, 48, 72, 96, 120, 144 and 168 h after mycelia inoculation and expressed as CAT activity (µmol H_2O_2/min mg^{-1} protein).

The highest catalase activity in roots was obtained at 96 h and 72 h with 9.93 and 4.63 µmol H_2O_2/min mg^{-1}protein after pathogen inoculation for cultivar PJ-1430 and SU-1080, respectively, which was 176.6% and 113% higher in inoculated plants as compared to the control plants (Fig. 3). The catalase activity was 2.8 and 2 fold higher in cultivar PJ-1430 and SU-1080, respectively, in pathogen inoculated plants as compared to the catalase activity at 0 h.

Our findings are similar with the earlier studies who reported that the enhanced catalase activity was observed in resistant variety of *Capsicum annuum* against the pathogen *Phytophthora capsici* L. in comparison to the susceptible one [21]. Similarly, induction of CAT has also been observed in nodulated soybean roots colonized by *Glomus mosseae* [22].

The CAT activity in leaves (Fig. 4) was 6.54 µmol H_2O_2/min mg^{-1} protein and 5.28 µmol H_2O_2/min mg^{-1} protein in cultivar PJ-1430 and SU-1080 which was 2.5 and 1.7 fold higher than the control at 0 h. Similarly, infection by *Fusarium oxysporum* significantly increased CAT activity in leaves of tomato plants at different stages of growth as compared with non-infected control plants [23].

Overall, on comparing the leaves and roots of sorghum plants the CAT activity was higher in roots as compared to leaves. Our study indicates that the antioxidant enzyme CAT is actively involved in imparting resistance to charcoal rot disease of sorghum. It was observed that upon inoculation CAT expression was higher in cultivar PJ-1430 as compared to SU-1080. This may inhibit the growth of pathogen by suppressing attempted invasion there by imparting resistance to sorghum against *M. phaseolina*.

3.3 Superoxide dismutase (SOD) activity in root and leaves of *S. bicolor* infected with *M. phaseolina*

The effect of *M. phaseolina* on SOD activity in both the cultivars of *S. bicolor* is shown in (Fig. 5-6). The SOD activity was higher in root and leaf samples as compared to control after pathogen elicitation. The SOD activity was determined in terms of SOD activity NBT reduced mg^{-1}protein.

The maximum SOD activity obtained at 96 h in root 6.17 NBT reduced mg^{-1}protein and 3.77 NBT reduced mg^{-1}protein which was 3 and 2.5 fold higher in the pathogen inoculated plants of cultivar PJ-1430 and SU-1080, respectively, as compared to control (Fig. 5).

Our results are similar to the results reported by [24] who observed induced SOD activity in roots of *Pinus massoniana* after infection with *Pislithus tinctorius.* Similarly, significant increase in roots of chickpea plant following *Fusarium oxysporum* interaction [25]. Further, [26] reported the differential induction of superoxide dismutase in downy mildew-resistant and susceptible genotypes of pearl millet (*Pennisetum glaucum*) on inoculation with *Sclerospora graminicola*. *Gossypium hirsutum* varieties resistant to verticillium wilt (*Verticillium dahliae*) showed increased SOD activity, while susceptible ones showed slightly decreased activity as compared to resistant varieties [27]. The potential accumulation of H_2O_2 by SOD activity may have several important effects on the host-pathogen interaction. Hence, an increase in SOD activity was observed in resistant genotypes after inoculation. Upon inoculation in resistant genotypes H_2O_2 accumulates due to SOD activity. This may inhibit the growth and viability of the pathogen, directly suppressing attempted invasion. This in turn may impart resistance to sorghum against root rot disease in resistant genotypes.

15 days old sorghum leaves indicates a higher SOD activity in pathogen inoculated plants compared to control plants. The maximum SOD activity in pathogen inoculated sorghum plants occurs at 96 h and 72 h which was 2 and 1.4 folds higher in cultivar PJ-1430 and SU-1080, respectively, as compared to 0 h inoculated plants (Fig. 6).

However, with further increase in inoculation time beyond 96 h, no further increase in the SOD activity is observed, rather a gradual decrease up to 168 h was observed (Fig. 5-6). Further the SOD activity is higher in PJ-1430 as compared to cultivar SU-1080. This is in agreement with results reported by [28] that in SOD activity in leaves of barley plants increased in pathogen (*Blumeria graminis*) inoculated plants than the control ones. Similarly, increase in the SOD activity in leaves of *Nicotiana benthamiana* plant infected with *Pepper mild mottle* virus [29].

Superoxide dismutase (SOD) plays an important role in antioxidant defense system. It is known to catalyze the production of hydrogen peroxide (H_2O_2) by scavenging superoxide radicals ($O^{2\cdot-}$). The production of reactive oxygen species (ROS) is one of the earliest cellular responses following successful pathogen recognition. ROS was considered as the first defense line against pathogen and may act as direct antimicrobial agent against phytopathogen attack. The SOD, catalyzing the dismutation reaction of the free radicals to the hydrogen peroxide (H_2O_2), seems to play a key role in the apparition and extension of the necrotic

reaction. The induction of SOD activity was frequently reported in the plants in response to pathogen invasion and constitutes a reaction often associated to the plant resistance [30].

3.4 Ascorbate peroxidase (APX) activity in leaves and root of *S. bicolor* infected with *M. phaseolina*

The APX activity of 15 days old plants are given in Fig. 7. The activity was expressed in terms of APX activity µmol Ascorbate/min mg^{-1} protein.

APX activity in the inoculated plants of both the cultivars was significantly higher than that of the control plants. In cultivar PJ-1430, APX activity was higher than in cultivar SU-1080 at the all-time point investigated, in root samples. The level of APX reached its maximum (4.35 µmol Ascorbate/min mg^{-1}protein) at 72 h in the inoculated PJ-1430 cultivar, which was higher than that of SU-1080 (2.52 µmol Ascorbate/min mg^{-1}protein) at this time point (Fig. 5). Similarly, the increased production of APX in tomato root inoculated with *F. oxysporum* counteracted the higher MDA and H_2O_2 levels and prevents oxidative damage of root tissues [31]. Ascorbate peroxidase might be responsible for the fine modulation of ROS for signaling [32].

The APX activity in leaves of inoculated plants was (2.99 µmol Ascorbate/min mg^{-1} protein) and (1.80 µmol Ascorbate/min mg^{-1} protein) at 72 h which was 2 and 1.3 folds higher in PJ-1430 and SU-1080 respectively than the control plants. After that a continuous decline was observed in the activity in both the cultivars but the decline was more pronounced in SU-1080 (Fig. 8).

[33] observed an enhanced activity of APX in muskmelon plants inoculated with *Colletotrichum lagenarium*, and the level of APX activity was significantly higher in the resistant cultivar. Moreover, the higher levels of APX were associated with the partial resistance of tomato plants to *Rhizoctonia solani* [34]. These reports are in agreement with the data from the present study, which show that the APX activity was maintained at a higher level in the leaves of the resistant sorghum plants than in susceptible plants during *M. phaseolina* infection process.

APX is thought to play the most essential role in scavenging ROS and protecting cells in higher plants [35]. Among peroxidases, ascorbate peroxidases are well known for their role in H_2O_2 detoxification in plant [36]. Infection of sweet orange leaves by *Xanthomonas axopodonis* subsp citri showed increase in activity of ascorbate peroxidase activity [37]. The change in antioxidant enzyme activity in tomato leaves infected by *Fusarium* was reported [31].

3.5 Glutathione reductase (GR) activity in roots and leaves of *S. bicolor* infected with *M. phaseolina*

The GR activity of 15 days old plants are given in Fig. 9-10. The activity was expressed in terms of GR activity µmol NADPH/min mg^{-1} protein.

S. bicolor cultivars PJ-1430 and SU-1080 showed a differential response in GR activity with the increase in the time interval of pathogen inoculation as compared to control. In cultivar PJ-1430, GR activity increased gradually upto 96 h, after that the GR activity gets reduced but was still higher than control. On the other hand in cultivar SU-1080, the GR activity gets induced and reaches to maximum at 72 h and then decreased gradually till 168 h. GR activity reached its maximum to 3.18 µmol NADPH/min mg^{-1} protein in PJ-1430 whereas the cv. SU-1080 leveled upto 2.76 µmol NADPH/min mg^{-1} in root samples of *S. bicolor* (Fig. 9). The results are in agreement with [25] who reported the induction of GR activity in *Cicer arietinum* on inoculation with *Fusarium oxysporum*. GR activity significantly increased in the roots of chickpea plant. Increased activity of GR, a H_2O_2 detoxifying enzyme, observed in our experiments, is likely to have been induced by elevated levels of H_2O_2, as a response to oxidative stress. Similarly, the same pattern of induction of GR was observed in plant roots colonized with *Piriformospora indica* and *Fusarium verticillioides-Hordeum vulgare* interactions and it was suggested that the higher GR activity maintains an enhanced level of reduced glutathione which is involved in maintaining antioxidant capacity [38].

Similarly, the GR activity in leaves of both cultivars was increased at 96 h and then a gradual decline was observed. GR activity was highest in PJ-1430 2.84 µmol NADPH/min mg^{-1} and in the susceptible cultivar the value was 2.07 µmol NADPH/min mg^{-1} which was 2.4 and 1.5 folds higher than the control in both the cultivars respectively (Fig. 10). Similar results were reported by [39] who reported the increase in the GR activity in wheat plants inoculated with *Pyricularia oryzae* at 96 h post inoculation.

Further, the GR activity in leaves of maize plants increased upto 2 folds in maize plants inoculated with *Piriformospora indica* [40]. Similarly, the GR activity was measured in the two barley cultivars, resistant and sensitive against *B. graminis* which leads to an increase in the GR activity in the tolerant cultivar than the sensitive one [41]. Inoculation of tomato leaves with *Botrytis cinerea* led to markedly increase in the GR activity [42].

Overall, on comparing the leaves and roots of sorghum plants the GR activity was found to be higher in roots as compared to leaves. Similar results were reported by [25] who reported the induction of GR activity in *Cicer arietinum* on inoculation with *Fusarium oxysporum*.

4. Figures

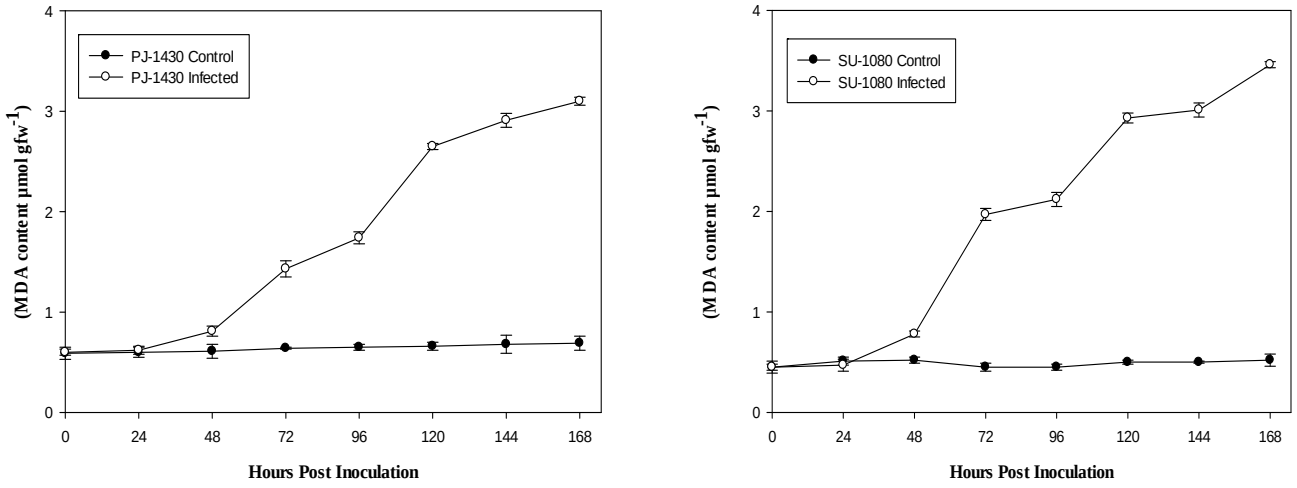


Fig. 1: Changes in the MDA content in control and pathogen (*Macrophomina phaseolina*) inoculated 15 days old *Sorghum bicolor* plants root samples of cultivar viz. PJ-1430 (a) and SU-1080 (b).

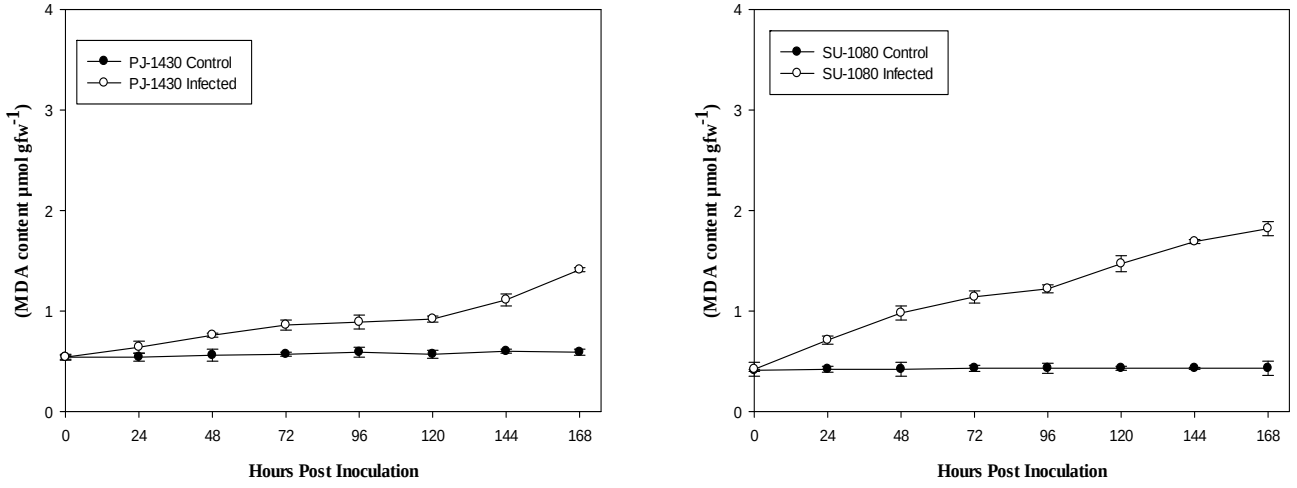


Fig. 2: Changes in the MDA content in control and pathogen (*Macrophomina phaseolina*) inoculated 15 days old *Sorghum bicolor* plants leaf samples of cultivar viz. PJ-1430 (a) and SU-1080 (b).

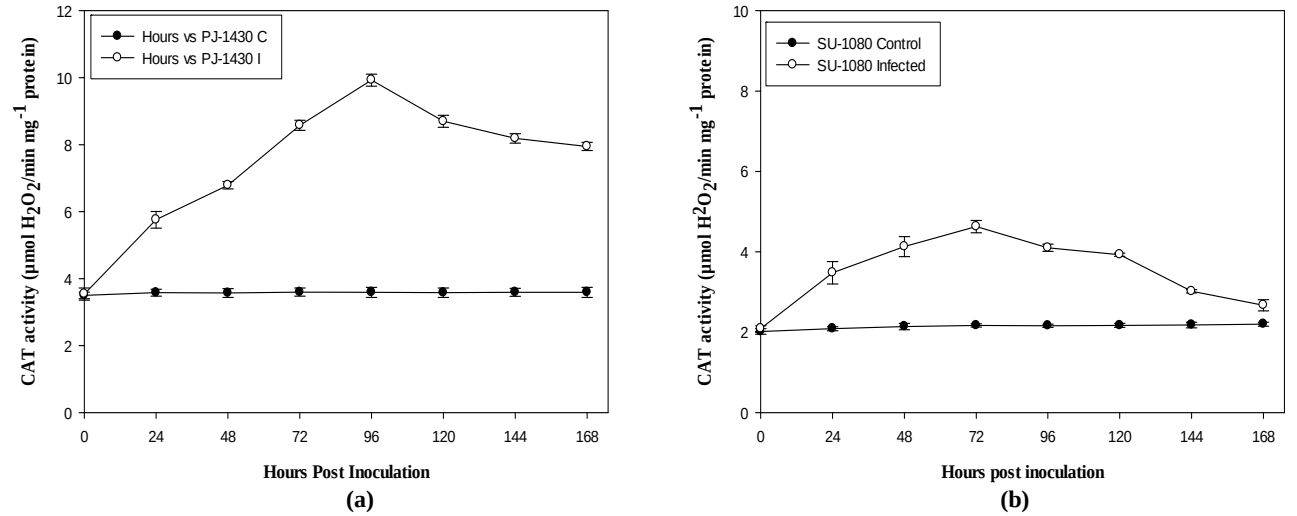
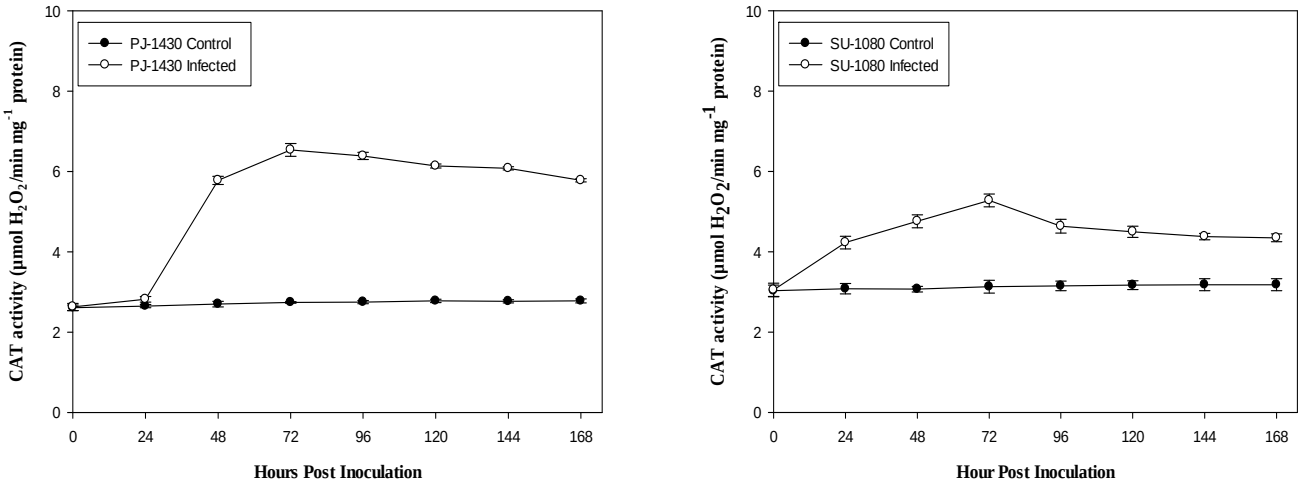
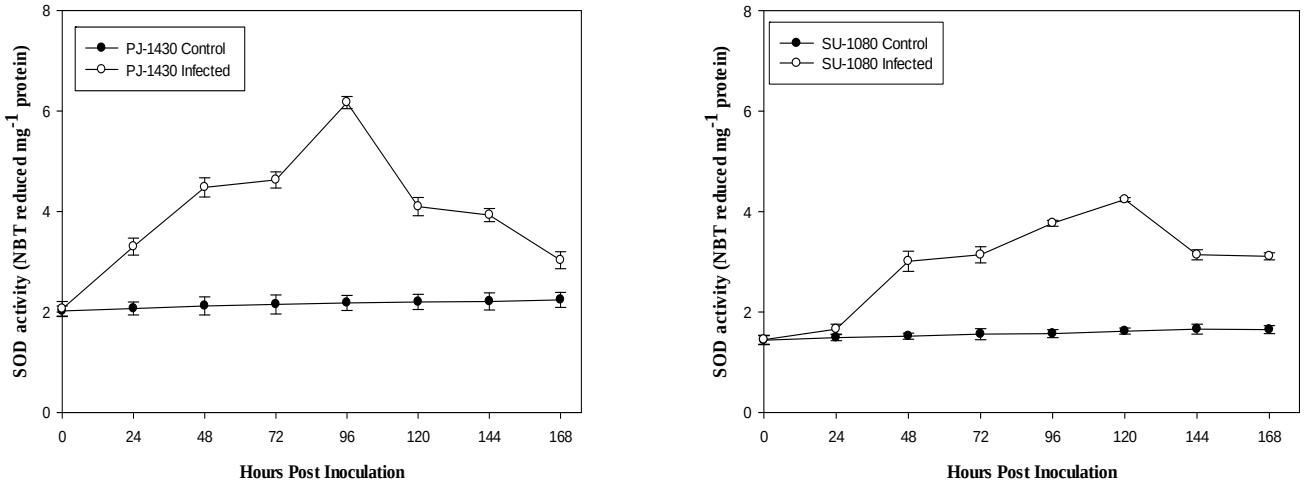


Fig. 3: Changes in the CAT activity in control and pathogen (*Macrophomina phaseolina*) inoculated 15 days old *Sorghum bicolor* plants root samples of cultivar viz. PJ-1430 (a) and SU-1080 (b).



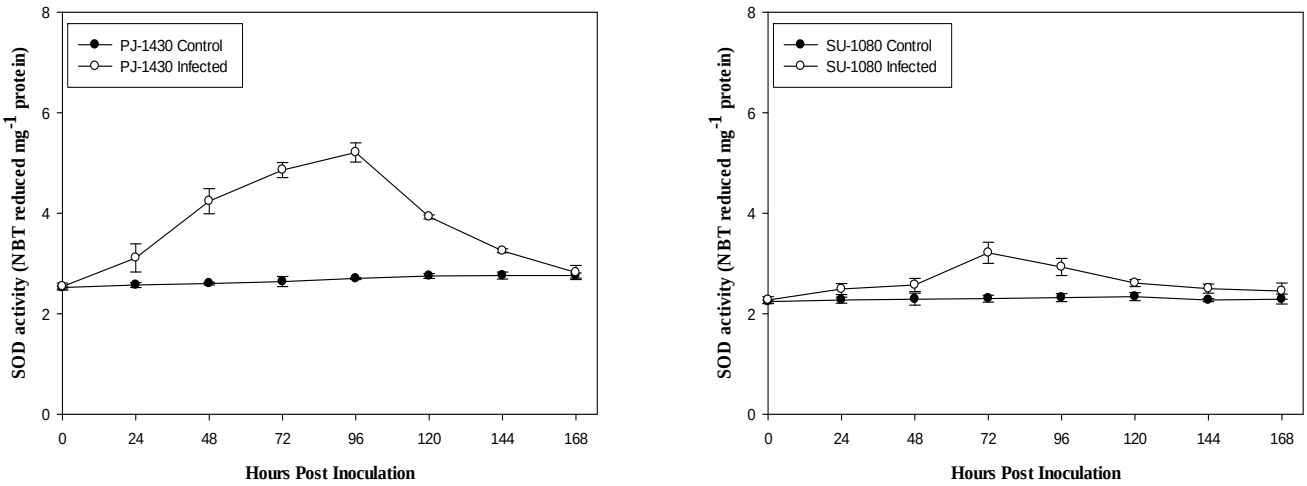
(a) **(b)**

Fig. 4: Changes in the CAT activity in control and pathogen (*Macrophomina phaseolina*) inoculated 15 days old *Sorghum bicolor* plants leaf samples of cultivar viz. PJ-1430 (a) and SU-1080 (b).



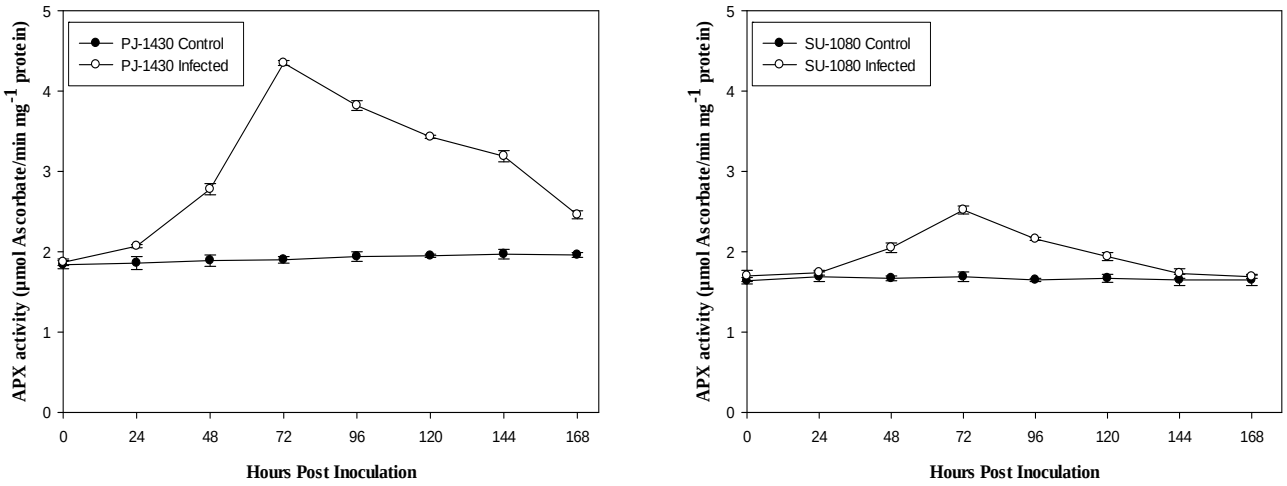
(a) **(b)**

Fig. 5: Changes in the SOD activity in control and pathogen (*Macrophomina phaseolina*) inoculated 15 days old *Sorghum bicolor* plants root samples of cultivar viz. PJ-1430 (a) and SU-1080 (b).



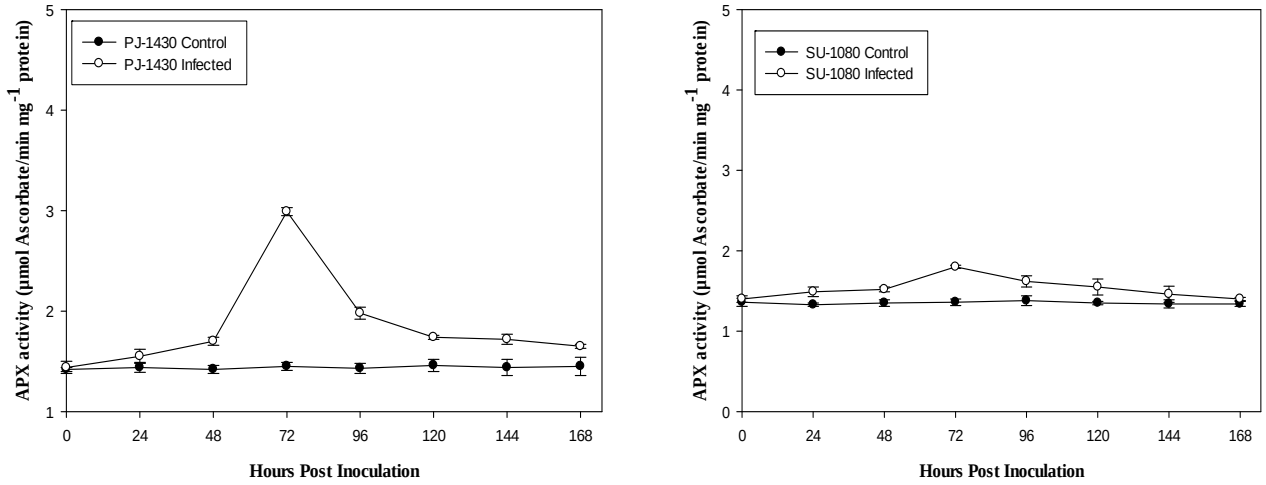
(a) **(b)**

Fig. 6: Changes in the SOD activity in control and pathogen (*Macrophomina phaseolina*) inoculated 15 days old *Sorghum bicolor* plants leaf samples of cultivar viz. PJ-1430 (a) and SU-1080 (b).



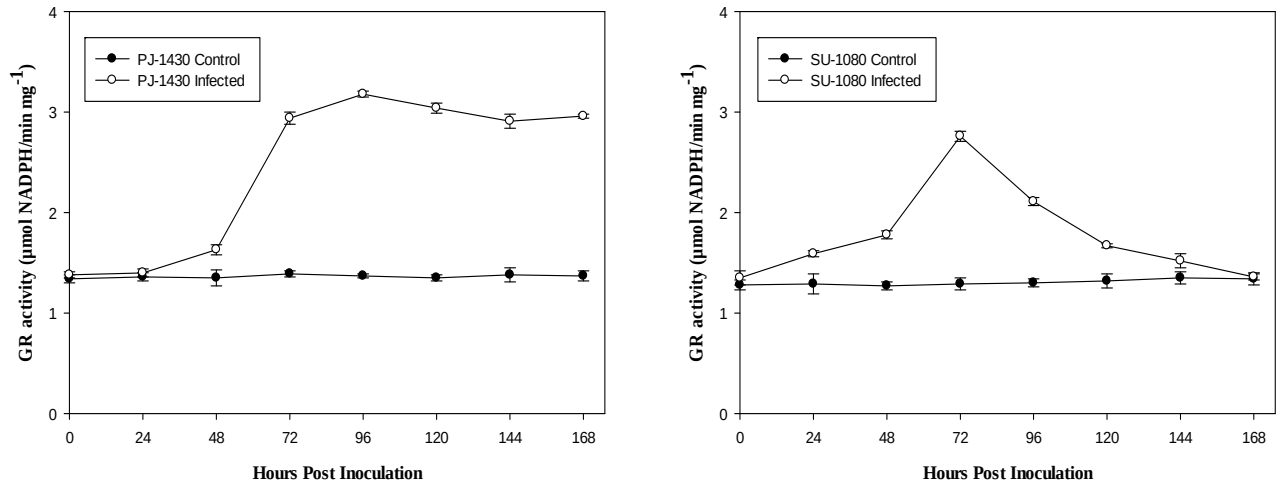
(a) **(b)**

Fig. 7: Changes in the APX activity in control and pathogen (*Macrophomina phaseolina*) inoculated 15 days old *Sorghum bicolor* plants root samples of cultivar viz. PJ-1430 (a) and SU-1080 (b).



(a) **(b)**

Fig. 8: Changes in the APX activity in control and pathogen (*Macrophomina phaseolina*) inoculated 15 days old *Sorghum bicolor* plants leaf samples of cultivar viz. PJ-1430 (a) and SU-1080 (b).



(a) **(b)**

Fig. 9: Changes in the GR activity in control and pathogen (*Macrophomina phaseolina*) inoculated 15 days old *Sorghum bicolor* plants root samples of cultivar viz. PJ-1430 (a) and SU-1080 (b).

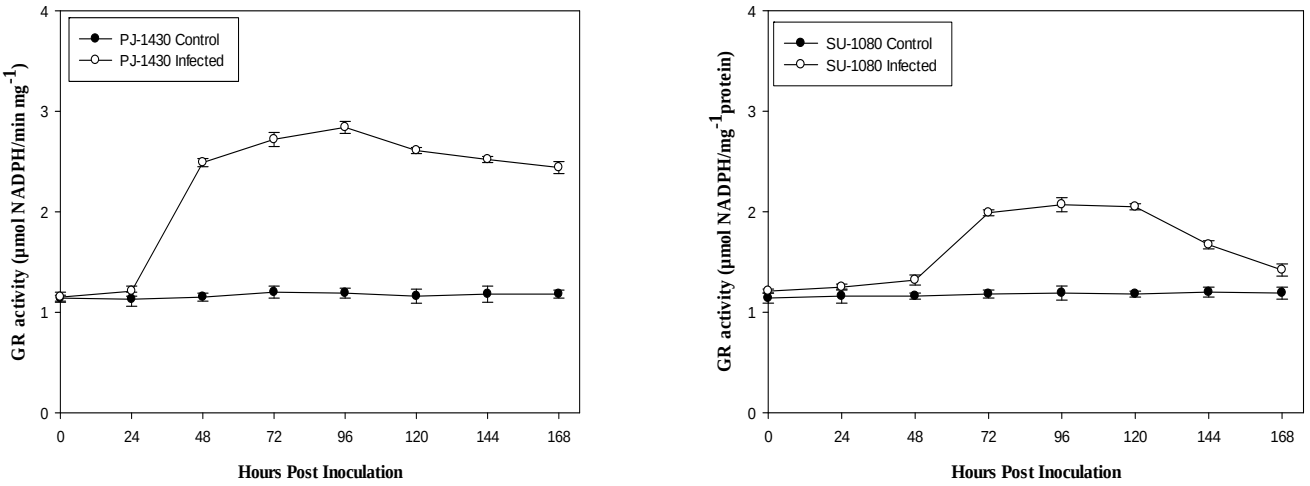


Fig. 10: Changes in the GR activity in control and pathogen (*Macrophomina phaseolina*) inoculated 15 days old *Sorghum bicolor* plants root samples of cultivar viz. PJ-1430 (a) and SU-1080 (b).

5. Conclusions:

MDA content increased with the progression of the infection time reflecting enhancement of lipid peroxidation. As a result of infection plants have evolved antioxidative machinery to combat oxidative stress. The activities of SOD, CAT, APX and GR increased to combat the effect of lipid peroxidation in sorghum. The induction of ROS-scavenging enzymes, such as superoxide dismutase (SOD), glutathione reductase (GR) and catalase (CAT) are the most common mechanism for detoxifying ROS synthesized during stress responses. Based on the above results it may be concluded that high activity of antioxidative enzymes of the *S. bicolor* appear to be important biochemical constituents in imparting resistance to *M. phaseolina* charcoal root rot disease.

6. Acknowledgements

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7. References

1. Das IK, Indira S, Annapurna A, Prabhakar, Seetharam N. Biocontrol of charcoal rot in sorghum by fluorescent *Pseudomonads* associated with the rhizosphere. Crop Protection, 2008; 27: 1407-1414.
2. Ma J, Hill CB, Hartman GL. Production of *Macrophomina phaseolina* conidia by multiple soyabean isolates in culture. Plant Disease, 2010; 94: 1088-1092.
3. Zveibil A, Mor N, Gnayem N, Freeman S. Survival, host-pathogen interaction, and management of *Macrophomina phaseolina* on strawberry in Israel. Plant Disease, 2012; 96: 265-272.
4. Wyllie TD. Charcoal rot. In: Sinclair JB, Backman PA. ed. Compendium of soybean diseases, 3rd Edn. American Phytopathological Society Press, St. Paul, MN, 1998, 114-118.
5. Gaige AR, Ayella A, Shuai B. Methyl jasmonate and ethylene induce partial resistance in *Medicago truncatula* against the charcoal rot pathogen *Macrophomina phaseolina*. Physiology and Molecular Plant Pathology, 2010; 74: 412-418.
6. Bashir MA, Malik BA. Disease of major pulse crops in Pakistan. Tropical Pest Management, 1988; 34: 309-314.
7. Muchero W, Ehlers JD, Close TJ, Roberts PA. Genic SNP markers and legume synteny reveal candidate genes underlying QTL for *Macrophomina phaseolina* resistance and maturity in cowpea. BMC Genomics, 2011; 12: 8.
8. Reuveni R, Nachmias A, Krikun J. The role of seed borne inoculum on the development of *Macrophomina phaseolina* on melon. Plant Disease, 1983; 67: 280-281.
9. Abawi GS, Pastor-Corrales MA. Root rots of beans in Latin America and Africa: diagnosis, research methodologies, and 129 management strategies. CIAT. Cali, Colombia 1990, 114.
10. Kanazawa S, Sano S, Koshiba T, Ushimaru T. Changes in antioxidative in cucumber cotyledons during natural senescence: Comparison with those during dark induced senescence. Physiologia Plantarum, 2000; 109: 211-216.
11. Iqbal U, Mukhtar T. Morphological and pathogenic variability among *Macrophomina phaseolina* isolates associated with mungbean (*Vigna radiate* L.) Wilczek from Pakistan. The Scientific World Journal, 2014; Article ID 950175, pp. 9.
12. Sharma I, Kumari N, Sharma V. Defense gene expression in *Sorghum bicolor* against *Macrophomina phaseolina* in leaves and root of susceptible and resistant cultivars. Journal of Plant Interactions, 2014; 9(1): 315-323.
13. De Vos CHR, Schat H, De Waal MAM, Vooijs R, Ernst WHO. Increased to copper-induced damage of the root plasma membrane in copper tolerant *Silene cucubalus*. Plant Physiology, 1991; 82: 523-528.
14. Beauchamp CH, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Annals of Biochemistry, 1971; 44: 276-287.
15. Aebi H. Catalase in vitro. Methods Enzymology, 1984. 105: 121-126.
16. Nakano Y, Asada K. Purification of ascorbate peroxidase in spinach chloroplasts: its inactivation in ascorbate depleted medium and reactivation by

monodehydroascorbate radical. Plant Cell Physiology, 1987; 28: 134-139.
17. Barata RM, Chapparro A, Chabregas SM, Gonzalez R, Labate CA, Azevedo RA, Sarath G, Lea PJ, Silva-Filho MC. Targeting of the soybean leghemoglobin to tobacco chloroplasts: Effects on aerobic metabolism in transgenic plants. Plant Science, 2000; 155: 193-202.
18. Shimzu N, Hosogi N, Hyon GS, Jiang S, Inoue K, Park P. Reactive oxygen species (ROS) generation and ROS induced lipid peroxidation are associated with plant membrane modifications in host cells in response to AK-toxin from *Alternaria alternate* Japanese pear pathotype. Journal of General Plant Pathology, 2006; 72: 6-15.
19. Melan MA, Dong X, Endara ME, Ausubel KR, Peterman TK. An *Arabidopsis thaliana* lipoxygenase gene can be induced by pathogen, abscisic acid, and methyl jasmonate. Plant Physiology, 1993; 101: 441-450.
20. Rasoulnia A Mehdi S, Hossein A, Naser A, Masoud F, Najafabadi S. Antioxidant activity and lipid peroxidation in response to citrus canker bacterial infection. International Journal of Fundamental and Science, 2013; 2(24): 1179-1184.
21. Koc E, Ustun AS. Influence of *Phytophthora capsici* L. inoculation on disease severity, necrosis length, peroxidase and catalase activity, and phenolic content of resistant and susceptible pepper (*Capsicum annuum* L.) plants. Turkish Journal of Biology, 2012; 36: 357-371.
22. Porcel R, Barea JM, Ruiz-Lozano JM. Antioxidant activities in mycorrhizal soybean plants under drought stress and their possible relationship to the process of nodule senescence. New Phytology, 2003; 157: 135-143.
23. Samia M, Khalla E. 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. Australian Journal of Basic and Applied Sciences, 2007; 1: 717-732.
24. Kong FX, Liu Y, Hu W, Shen PP, Zhou CL, Wang LS. Biochemical responses of the mycorrhizae in *Pinusm assoniana* to combined effects of Al, Ca and low pH. Chemosphere, 2000; 40: 311-318.
25. García-Limones C, Hervás A, Navas-Cortés JA, Jiménez-Daíz RM, Tena M. Induction of an antioxidant enzyme system and other oxidative stress markers associated with compatible and incompatible interactions between chickpea (*Cicer arietinum* L.) and *Fusarium oxysporum* f. sp. ciceris. Physiology and Molecular Plant Pathology, 2002; 61: 325-337.
26. Babitha MP, Bhat SG, Prakash HS, Shetty HS. Differential induction of superoxide dismutase in downy mildew-resistant and susceptible genotypes of pearl millet. Plant Pathology, 2002; 51: 480-486.
27. Zhu HQ, Song XX, Xing JS, Guo JC. Relationship between antioxidative system of cotton and its resistance to *Verticillium* wilt stress. Acta Agriculturae Boreal Sin, 1995; 10: 130-133.
28. Vanacker H, Carver TLW, Foyer CH. Pathogen-induced changes in antioxidant status of the apoplast in the barley leaves. Plant Physiology, 1998; 117(3): 1103-1114.
29. Hakamaoui A, Pérez-Bueno ML, García-Fontana B, Camejo D, Jiménez A, Sevilla F, Barón M. Analysis of the antioxidant response of *Nicotiana benthamiana* to infection with two strains of *Pepper mild mottle virus*. Journal of Experimental Botany, 2012; 1-10.
30. Jetiyanon K. Defensive-related enzyme response in plants treated with a mixture of *Bacillus* strains (IN937a & IN937b) against different pathogens. Biological Control, 2007; 42: 178-185.
31. Mandal S, Mitra A, Mallick N. Biochemical characterization of oxidative burst during interaction between *Solanum lycopersicum* and *Fusarium oxysporum* f. sp. Lycopersici. Physiology and Molecular Plant Pathology, 2008; 72: 56-61.
32. Grant JJ, Yun BW, Loake GJ. Oxidative burst and cognate redox signalling reported by luciferase imaging: identification of a signal network that functions independently of ethylene, SA and Me-JA but is dependent on MAPKK activity. The Plant Journal, 2000; 24**:** 569-582.
33. Ge Y, Guest DI, Bi Y. Differences in the induction of defense responses in resistant and susceptible muskmelon plants infected with *Colletotrichum lagenarium*. Journal of Phytopathology, 2014; 162: 48-54.
34. Nikraftar F, Taheri P, Rastegar MF, Tarighi S. Tomato partial resistance to *Rhizoctonia solani* involves antioxidative defense mechanisms. Physiology and Molecular Plant Pathology, 2013; 81: 74-83.
35. Gill SS, Tuteja N. Reactive oxygen species and anti-oxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry, 2010; 48: 909-930.
36. Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annual Review in Plant Biology, 2004; 55: 373-399.
37. Kumar N, Ebel RC, Roberts PD. Superoxide dismutase activity in kumquat leaves infected with *Xanthomonas axonopodis* pv. citri. Journal of Horticulture Science and Biotechnology, 2011; 86: 62-68.
38. Waller F, Baltruschat H, Achatz B, Becker K, Fischer M, Fodor J, Heier T, Huckelhoven R, Neumann C. The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. Proceedings of the National Academy of Sciences. USA, 2005; 102: 13386-13391.
39. Debona D, Rodrigues FÁ, Rios JA, Nascimento KJT. Biochemical changes in the leaves of wheat plants infected by *Pyricularia oryzae*. Phytopathology, 2012; 102: 1121-1129.
40. Kumar M, Yadav V, Tuteja N, Johri AK. Antioxidant enzyme activities in maize plants colonized with *Piriformospora indica*. Microbiology, 2009; 155(3): 780-790.
41. El-Zahaby HM, Gullner G, Király Z. Effect of powdery mildew infection of barley on the ascorbate-glutathione cycle and other antioxidants in different host-pathogen interactions. Phytopathology, 1995; 85: 1225-1230.
42. Kuniak E, Sklodowska M. Ascorbate, glutathione and related enzymes in chloroplasts of tomato leaves infected by *Botrytis cinerea*. Plant Science, 2001; 160(4): 723-731.