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Progressive impact of alternaria blight on antioxidants enzyme of mustard leaves after infection of *Alternaria brassicae* to induce resistance

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Abstract

Mustard (brassica) is second most important oil seed crop which belongs to family brassicaeae. It contributes 17% in domestic oil production. Alternaria is a very lethal disease which is caused by *Alternaria brassicaeae*. It causes 47% yield losses. *Brassica napus* is infected by *Alternaria* blight which is responsible to cause variations in its biomolecules and antioxidant profile. The research was undertaken to study variation in biochemistry of resistance associated enzymatic profile of brassica germplasm. Pot experiment was designed on such fifteen varieties in greenhouse. Inoculum was prepared to inoculate these varieties and healthy leaves were collected along with inoculated showing symptoms of disease. Collected samples were analyzed using spectrophotometer. Results showed that Rapid decrease or reduction in catalase and SOD activity was observed in inoculated leaves of moderately susceptible varieties Punjab canola (0.0373) and Faisal canola (0.069), (0.211 and 0.097) to (0.077 and 0.082) respectively. Overall varieties depicted increasing trend but commonly H_2O_2 and POD in moderately susceptible cultivars was slightly decreased from (0.0587, 0.2350) to (0.0530, 0.1973). In contrast drastic increase was found in highly susceptible varieties while it was obvious that behavior of susceptible to highly susceptible lines was almost same. Highly susceptible lines showed slight decrease comparable to susceptible one. Protein activity was found to increase in inoculated leaves of all varieties. Phenolic activity in moderately Susceptible lines (Punjab Canola and Faisal Canola) rapidly and suddenly climbed up followed by susceptible cultivars. Data showed the smooth change protein and phenolic activity. In highly susceptible varieties (DGL, BSA and Toria selection A) phenolic level was (1.451, 1.344, 1.142) as compared to uninoculated leaves having (0.961, 0.816, 2.273 and 1.344) protein. It can be concluded that bio- antioxidant profile variations are useful tool to serve as effective marker for resistant germplasm documentation.

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Introduction

Mustard (*Brasicca napus*) is the 2nd largest and important oil seed crop grown in Pakistan and it belongs to the family *brassicaeae* (Christopher *et al*., 2005). Presence of beta-carotene, vitamin C and fibers make it more precious and nutritionally important element. It was firstly originated from South China, Europe and Canada and is cultivated in tropical and subtropical regions all over the world. Worldwide it is grown on an area of 37.0 m hectares with production of 63.6 million tones (Asif *et al*., 2017-18); Singh *et al*., 2014) while in Pakistan, it is cultivated on 193.5 thousand hectares with the production of 182 thousand tons in 2014-2015 (PODB, 2015). Mostly mustard is best growing in irrigated areas but only twenty five percent of it is cultivated in rain fed areas. This crop is contributing 18-20% in edible oil production of Pakistan while other 80% is fulfilled by import from other countries (Abbas *et al*., 2009; Asif *et al.,* 2017, 2018). This crop undergoes to various stresses but *Alternaria* Blight disease is the most destructive one in the entire world. Among important *Alternaria* species *A. brassicicola*, *A. brassicae* and *A. japonica* are responsible for significant yield and quality losses in brassica but *A. brassicae* is entirely damaging for mustard crop (Verma and Saharan, 1994). Various research reports quoted up to 47% yield losses against thus mustard disease (Meena *et al*., 2010). This pathogen lowers the photosynthesis rate, causes shattering of premature pod, increases senescence, and production of shriveled seeds are characteristic symptoms of this disease (Shresta *et al*., 2000*)*. These symptoms appear on the older leaves first, later on stems of seedlings and on premature pods. On leaves first tan colour spots appear which gradually increase and interlink to form a lesion which reduces the photosynthetic rate (Kubota *et al.,* 2003*)*. Similarly, at seedling stage, dark color lesions appear on stem while brown to black spots appear on hypocotyl which lowers plant growth (Valkonen and Koponen, 1990). Pathogens can survive 25 to 35°C but 30°C is the optimum temperature for the mycelium growth. Mycelium growth and sporulation increases with increase in relative humidity (Meena *et al.,* 2008).

Alternaria brassicae attack induces various type of biochemical alterations in plant. These alterations are very helpful to study the biochemical mechanisms and interaction between the host plant-pathogen. Hypersensitive response produced by the activation of Oxygen species through a mechanism known as oxidative burst enable plant to defend itself against the sudden attack of pathogen. Virulence factors released by the pathogen are recognized by plant through their receptor molecules. These molecules are necessary for the quick activation of plant defense like hypersensitive response which blockage the further transmission of pathogen from the point of infection to other plant parts and death of surrounding tissues. It causes the restriction of water and nutrients of pathogen which leads to a battle for its own survival and ultimately to pathogen death and plant life. Activities of antioxidant enzymes as peroxidase (PO), superoxidase dismutase (SOD), catalase (CAT), polyphenol oxidase (PPO), ascorbate peroxidase (APX) and metabolites like sugars, proteins and free amino acids fluctuate after the attack of *Alternaria blight* on Mustard and most probably increased (Abedi and Pakniyat, 2010). It was also observed that when *Alternaria* attack on mustard plants an increase in fatty acids (Ashraf *et al*., 1986) and phonelic contents (Kumar and Chauhan, 2005; Chattopadhyay, 2011) while reduction in chlorophyll contents take place (Barka and El-Matty, 2008). During the infection, both *Alternaria* generate and give out phytotoxins that belong to the host-specific toxins Parada *et al*., 2008, Wight *et al.,* 2009). Cruciferous plants are susceptible to infection and symptoms produced when attacked by phytotoxins (AB and ABR toxins) produced by *Alternaria brassicicola* and *Alternaria brassicae* respectively (Parada *et al*., 2008). production of lignin and phynylealanine ammonia lyase (PAL) catalyzed by peroxidase enzymes take part in the synthesis of plant weapons like phytoalexins and phenolic compounds involved in plant defense against the pathogen (Karthikeyan *et al.,* 2009). Disproportion of toxic superoxide free radical (O^{2-}) to hydrogen peroxide (H_2O_2) and oxygen (O_2) is catalyzed by super oxide dismutase enzyme. H_2O_2 change the reduction-oxidation status of the surrounding cells and gives an antioxidative response by acting as a signal of oxidative stress (Hung *et al.,* 2005).

Host resistance and susceptibility depends upon the complex networking of molecular and cytological events which are conferred by interaction between host and pathogen. Peroxidase and other isoenzyme are positively co-related to initiate resistance response (Nawar and Kuti, 2003). That's why it was dire need to observe these alterations of different biochemical compounds with varying disease incidence. Although, there are several pathogens present on the surface of plant which can produce similar type of symptoms and simple symptomology study is not enough to identify source of resistance. Present study of biochemical changes was aimed to identify the source of resistance and to analyze changes in concentrations of Catalases (CAT), Peroxidases (POD), Super oxide dismutase (SOD), Hydrogen peroxide (H_2O_2), total Phenolics and protein in uninoculated and inoculated leaves. And also explore the possibility of biochemical changes and biochemical markers for the identification of resistant source and defense of oilseeds Brassica against pathogen.

Materials and methods

This research was designed in Department of Plant Pathology, University of Agriculture Faisalabad research area. While fifteen rapeseed cultivars i.e DGL, Rainbow, CON II, Oscar, Punjab canola, Faisal canola, Excel, Cyclone, Shirale, Dunkeld, Legend, CON III, BSA, Bulbul 98, Toria Selection-A, were brought from near research station (AARI), Faisalabad. Recommended method was used to sow such varieties using Randomized Complete Block Design while planting and row distance was maintained 25 and 75cm respectively and replication were kept three for each variety. All the cultural practices were performed to keep them hygienic. Healthy and infected diseased samples was collected from the field in separate brown bags, labeled with permanent marker and placed in polythene bags to store in cold temperature of (-20˚C) in refrigerator (PEL®) in laboratory for further biochemical analysis. Frozen samples were washed with tap water and then with distilled water. After air drying, 10mg leaves was weighed and cut into uniform small pieces for grinding.

Extraction buffer Na_2HPO_4, and NaH_2PO_4 was prepared. Leaves were grinded using this buffer in pestle and mortar. Extracted liquid was kept in eppendorf for further analysis. Centrifugation was carried out in "Table Top Centrifuge" machine before storing at 4˚C for 10-15 minutes. Residual material was discarded and only filtered supernatant was stored at 4˚C in refrigerator (*PEL,* PRGD-145). Later enzyme test was conducted.

Superoxide dismutase

Superoxide dismutase concentration was recorded by its ability to inhibit the photo reduction of Nitro-blue-tetrazolium (Shahid *et al.*, 2012). For this purpose, standard doses of phosphate buffer with pH 7.8, NBT, methionine, TritonX, Ribo-flavin were used to prepare reaction mixture by mixing in a sequence described. Resultant mixture was poured in test tubes for UV treatment under wooden chamber for fifteen minutes. Plant leaves extract was mixed at the end of this treatment @ 50µl. In the very last, 100µl H_2O_2 and 100µl reaction solution was poured in ELISA plate (UltraCruz® ELISA Plate, 96-wells) by micropipets and Absorbance was recorded by Absorbance reader (BioTek, Model:800TS), at 560 nano meter (Shahid *et al.,* 2012).

Catalase and Peroxidase

Plant liquid extract of 100µl was mixed with standard phosphate buffer of pH 8.3, while recommended dose of H_2O_2 and 100µl enzyme extract were poured in test tube. The reaction was undertaken using 150µl samples on ELISA micro-plate (UltraCruz® ELISA Plate, 96-wells) and absorbance was taken at 240nm. However, to assess the Peroxidase pH of phosphate buffer was lowered to 7, and another reagent guaiacol with H_2O_2, were mixed to get reaction solution and sample was taken in eppendorf to mix it with leaf extract. 100-150µl samples was loaded on micro-plate (UltraCruz® ELISA Plate, 96-wells) and absorbance was taken at 470nm via Absorbance reader (BioTek, Model:800TS). One unit of catalase and Peroxidase activity was considered as an absorbance change of 0.01 unit's min^{-1} (Shahid *et al.,* 2012).

H_2O_2 Concentration

This enzyme analysis was carried out using fresh leaves either infected or healthy. Only 50mg fresh leaves were weighed and grinded using grinder within Trichloroacetic acid buffer and centrifuged at 12,000rpm for 15min at 4°C. filtered solution was treated with buffer i.e potassium phosphate (pH 7) and after that with potassium iodide. Resultant mixture kept in Digital incubator for 5 minutes and absorbance was taken at 390nm via Absorbance reader (BioTek, Model:800TS). The amount of H_2O_2 was indicated as µmol·g^{-1} FW (Velikova *et al.*, 2000).

Protein activity

40µL leaf extract with 160µL Bradford reagent was loaded on ELISA plate (Ultra Cruz® ELISA Plate, 96-wells) and absorbance was measured with the help of Absorbance reader (BioTek, Model:800TS) at 595nm (Bradford, 1976).

Phenolics activity

Reaction mixture for phenolics or total phenolics was made of 5ml of FC reagent mixed with 45ml of double distilled and 10g sodium carbonate mixed with water. 100µl leaf extract and 50µl FC reagent mixture was taken in eppendorph and shaken thoroughly. 150µl sample was loaded on micro plate (UltraCruz® ELISA Plate, 96-wells) to observe the absorbance at 765nm in Absorbance reader (BioTek, Model:800TS) (Shahid *et al.*, 2012).

Statistical analysis

Data obtained from field trials parameters was subjected to randomize complete block design (RCBD) as described by Steel *et al.*, 1997. Least significant difference (LSD) design was applied to determine the significant differences. All the statistical analysis was performed by using SAS statistical software (SAS institute, 1990).

Results and discussion

Catalase

Rapid decrease of catalase level can be observed in highly susceptible lines DGL, and BSA and Toria selection-A due to the attack of *A. Brassice*.

Similarly, Reduction in catalase activity was observed in inoculated leaves of moderately susceptible varieties Punjab canola (0.0373) and Faisal canola (0.069) as compared to the uninoculated leaves of moderately susceptible varieties which contain catalase enzyme respectively. In addition, susceptible cultivars (Rainbow, Con II, Oscar, Excel, Cyclone, Shirale, Dunkled, Legend, CON III and Bulbul 98) showing overall declining trend of CAT which ranges from (3.62, 3.61, 3.31, 3.82, 3.57, 3.62, 3.26, 3.42, 3.53and 3.70) respectively. DGL, BSA and Toria selection A were highly susceptible varieties which contain (3.67), (3.68) and (3.46) concentration of CAT in inoculated leaves as compared to the uninoculated leaves (3.38), (3.44) and (3.63) respectively. The trend of CON III, shirale and dunkld was deviating from normal behavior. Conversely speaking, decrease in catalase activity was indication of scavenging the hydrogen peroxide activity after infection (Table 1).

Table 1. Comparison of mean values of catalase activity (µ mol H_2O_2 mg^{-1} Protein) in uninoculated and inoculated leaves of Brassica varieties/lines.

Catalase (CATs) Activity (µ mol H_2O_2 mg^{-1} Protein)			
Genotype	Response	Uninoculated	Inoculated
DGL	HS	3.671 c	3.387 h
Rainbow	S	3.621 c	3.685 bc
CON II	S	3.611 h	3.525 f
Oscar	S	3.316 f	3.506 f
Punjab canola	MS	3.556 ab	3.594 e
Faisal canola	MS	3.780 e	3.711 ab
Excel	S	3.828 i	3.263 i
Cyclone	S	3.572 cd	3.506 f
Shiralee	S	3.620 a	3.650 cd
Dunkeld	S	3.265 b	3.732 a
Legened	S	3.422 h	3.381 h
CON III	S	3.532 g	3.736 a
BSa	HS	3.688 f	3.447 g
Bulbul 98	S	3.701 i	3.695 b
Toria selection A	HS	3.462 a	3.631 i

Hydrogen peroxide

All the varieties were depicting increasing trend after infection to overcome the infection of pathogen. Concentration of H_2O_2 in moderately susceptible cultivars (Punjab canola and Faisal canola) slightly decreased from (0.0587, 0.2350) to (0.0530, 0.1973) respectively. In susceptible varieties there was a drastic increase in inoculated leaves as compared to uninoculated leaves.

Drastic increase was also found in highly susceptible varieties (DGL, BSA and Toria selection A) which range from (0.1143, 0.2880 and 0.1173) to (0.1283, 0.2350 and 0.1310) respectively. Very rapid increase in hydrogen peroxide was obivious in Toria selection A. Similarly, Con III and Shirale were demonstrating variable behviour as compared to other susceptible lines. They showed sudden and rapid increase in this enzyme level (Table 2).

Table 2. Comparison of mean values of H_2O_2 Activity (μ mol g^{-1} fw) in uninoculated and inoculated leaves of Brassica varieties/lines.

Hydrogen peroxide (H_2O_2) Activity (μ mol g^{-1} fw)			
Genotype	Response	Uninoculated	Inoculated
DGL	HS	0.114 g	0.128 g
Rainbow	S	0.044 m	0.056 i
CON II	S	0.098 j	0.258 a
Oscar	S	0.138 e	0.178 e
Punjab canola	MS	0.058 l	0.053 i
Faisal canola	MS	0.235 b	0.197 d
Excel	S	0.108 h	0.128 g
Cyclone	S	0.104 i	0.11 h
Shirale	S	0.074 k	0.225 c
Dunkeld	S	0.106 i	0.153 f
Legend	S	0.178 c	0.195 d
CON III	S	0.152 d	0.256 a
BSA	HS	0.288 a	0.235 b
Bulbul 98	S	0.113 g	0.197 d
Toria Selection A	HS	0.117 f	0.131 g

Peroxidase

Level of POD in moderately susceptible cultivars (Punjab canola and Faisal canola) was almost parallel to slightly increases from (0.138, 0.058) to (0.053, 0.197) respectively. If Common trend line is drawn, it will be obvious that behavior of susceptible to highly susceptible lines was almost same as it was increasing with few exceptions.

In susceptible varieties (Rainbow, CON II, Oscar, Excel, Cyclone, Shirale, Dunkeld, Legend, CON III and Bulbul 98) the amount of POD in inoculated leaves was (0.056, 0.258, 0.178, 0.197, 0.128, 0.11, 0.225, 0.153, 0.195, 0.256, 0.197) as compared to uninoculated leaves (0.044, 0.098, 0.138, 0.108, 0.104, 0.074, 0.106, 0.152, 0.113). Level of peroxidase enzyme in inoculated and uninoculated leaves of highly susceptible varieties like (DGL, BSA and Toria selection A) was (0.114, 0.128, 0.288, 0.235, and 0.117, 0113) respectively (Table 3).

Table 3. Comparison of mean values of peroxidase (POD) activity (μ mol H_2O_2 mg^{-1} Protein) in uninoculated and inoculated leaves of Brassica varieties/lines.

Peroxidase (POD) Activity (μ mol H_2O_2 mg^{-1} Protein)			
Genotype	Response	Uninoculated	Inoculated
DGL	HS	0.114 g	0.128 g
Rainbow	S	0.044 m	0.056 i
CON II	S	0.098 j	0.258 a
Oscar	S	0.138 e	0.178 e
Punjab canola	MS	0.058 l	0.053 i
Faisal canola	MS	0.235 b	0.197 d
Excel	S	0.108 h	0.128 g
Cyclone	S	0.104 i	0.110 h
Shiralee	S	0.074 k	0.225 c
Dunkeld	S	0.106 i	0.153 f
Legened	S	0.178 c	0.195 d
CON III	S	0.152 d	0.256 a
BSA	HS	0.288 a	0.235 b
Bulbul 98	S	0.113 g	0.197 d
Toria selection A	HS	0.117 f	0.131 g

Superoxidase dismutase

Level of POD in moderately susceptible cultivars (Punjab canola and Faisal canola) was almost parallel to slightly increases from (0.138, 0.058) to (0.053, 0.197) respectively. If Common trend line is drawn, it will be obvious that behavior of susceptible to highly susceptible lines was almost same as it was increasing with few exceptions.

Table 4. Comparison of mean values of (SOD) activity (μ mol H_2O_2 mg^{-1} Protein) in uninoculated and inoculated leaves of Brassica varieties/lines.

(SOD) Activity (μ mol H_2O_2 mg^{-1} Protein)			
Genotype	Response	Uninoculated	Inoculated
DGL	HS	0.097 h	0.048 h
Rainbow	S	0.088 ij	0.076 f
CON II	S	0.062 k	0.044 i
Oscar	S	0.136 g	0.115 b
Punjab canola	MS	0.211 d	0.077 f
Faisal canola	MS	0.097 h	0.082 e
Excel	S	0.157 f	0.119 a
Cyclone	S	0.093 hi	0.063 g
Shiralee	S	0.084 j	0.024 l
Dunkeld	S	0.184 e	0.096 c
Legened	S	0.059 k	0.041 j
CON III	S	0.391 c	0.082 e
BSA	HS	0.886 b	0.091 d
Bulbul 98	S	0.05 l	0.033 k
Toria selection A	HS	0.98 a	0.096 c

All the verities were showing decreasing sequence of events in brassica varieties when comparing inoculated and uninoculated ones.

Amount of SOD in moderately susceptible cultivars (Punjab Canola and Faisal Canola) decreased from (0.211 and 0.097) to (0.077 and 0.082) respectively while in susceptible cultivars (Rainbow, CON II, Oscar, Excel, Cyclone, Shirale, Dunkeld, Legend, CON III and Bulbul 98) amount of SOD ranges from (0.088, 0.062, 0.136, 0.157, 0.093, 0.084, 0.184, 0.059, 0.391 and 0.05) to (0.076, 0.044, 0.115, 0.119, 0.063, 0.024, 0.096, 0.041, 0.082 and 0.333) respectively. In highly susceptible verities (DGL, BSA and Toria Selection A) amount of SOD decreased (0.097, 0.886 and 0.98) to (0.048, 0.091 and 0.096) respectively. Highly susceptible lines were showing slight decrease comparable to susceptible one. Moderately susceptible lines depicted significant scavenging activity (Table 4).

Protein

Amount of protein activity was found to increase in inoculated leaves of all varieties as compared to the uninoculated leaves due to the pathogen attack. Protein activity in moderately susceptible cultivars (Punjab canola and Faisal canola) amount of protein decreases from (3.4527 and 2.0113) to (3.557 and 2.321) respectively. In inoculated leaves of susceptible variety (Rainbow, CON II, Oscar, Excel, Cyclone, Shirale, Dunkeld, Legend, CON III and Bulbul 98) amount of protein increased (2.993, 2.883, 2.636, 2.8663, 2.4283, 2.612, 1.987, 3.542, 3.0767 and 2.627) to (3.0483, 2.9853, 2.7917, 3.5477, 2.7377, 2.9487, 2.6040, 2.6180, 2.6947 and 2.4563) respectively. In highly susceptible varieties (DGL.BSA and Toria selection A) protein activity was increased (1.986, 3.431 and 3.0287) to (3.346, 2.495 and 3.305) respectively (Table 5).

Phenolic (mg/g of leaves)

Over all the response of phenolic activity was variable from variety to variety in inoculated leaves compared to the uninoculated leaves due to the interaction of pathogen with host. Phenolic activity in moderately Suceptilelines (Punjab Canola and Faisal Canola) rapidly and suddenly climbing up from (1.8963, 2.4173) to (2.536, 2.293) respectively while in susceptible cultivars Rainbow, CON II, Oscar, Excel, Cyclone, Shirale, Dunkeld, Legend, CON III and Bulbul 98)) level of phenolic enhanced from (0.987, 2.517, 1.424, 1.917, 2.581, 1.442, 1.773, 1.134, 1.162, 2.273, and 1.246) to (1.043, 1.474, 2.216, 2.293, 1.571, 2.253, 2.865, 2.946, 0.733, 1.344, and 0.664) respectively. Data showed the smooth change in concentration. In highly susceptible varieties (DGL, BSA and Toria selection A) phenolic level was (1.451, 1.344, 1.142) while in uninoculated leaves have (0.961, 0.816, 2.273 and 1.344) amount of protein (Table 6).

Table 5. Comparison of mean values of protein activity (mg/g of leaves) in uninoculated and inoculated leaves of Brassica varieties/lines.

Protein Activity (μ mol H_2O_2 mg^{-1} Protein)			
Genotype	Response	Uninoculated	Inoculated
DGL	HS	1.986 n	3.346 b
Rainbow	S	2.993 f	3.049 c
CON II	S	2.883 g	2.985 cd
Oscar	S	2.636 i	2.791 e
Punjab canola	MS	3.452 b	3.557 a
Faisal canola	MS	2.011 m	2.321 i
Excel	S	2.866 h	3.547 a
Cyclone	S	2.428 l	2.737 ef
Shiralee	S	2.612 k	2.948 d
Dunkeld	S	1.987 n	2.604 g
Legened	S	3.542 a	2.618 g
CON III	S	3.076 d	2.694 f
BSA	HS	3.431 c	2.495 h
Bulbul 98	S	2.627 j	2.456 h
Toria selection A	HS	3.028 e	3.305 b

Table 6. Comparison of mean values of phenolics activity (mg/g of leaves) in uninoculated and inoculated leaves of Brassica varieties/lines.

Phenolics Activity (μ mol H_2O_2 mg^{-1} Protein)			
Genotype	Response	Uninoculated	Inoculated
DGL	HS	1.451 de	0.961 l
Rainbow	S	0.987g	1.043k
CON II	S	2.517 a	1.474 h
Oscar	S	1.424 de	2.216 f
Punjab canola	MS	1.896 c	2.536 d
Faisal canola	MS	1.917 ab	2.293 j
Excel	S	2.581 a	1.571 g
Cyclone	S	1.442 de	2.253 e
Shirale	S	1.773 c	2.865 b
Dunkeld	S	1.134 fg	2.946 a
Legend	S	1.162 fg	2.605 c
CON III	S	1.507 d	0.733 m
BSA	HS	2.273 b	1.344 i
Bulbul 98	S	1.246 ef	0.664 n
Toria selection A	HS	1.142 fg	1.344 i

Discussion

Noctor and Fover, (1998) described that antioxidative enzymes are protectant of plants from the injury triggered by reactive oxygen species and other enzymes like Suoper Oxide Dismutase, catalase, GPX and APX via scavenging enzymes in plants. In vaccinated plants a substantial upsurge in Catalase activity was detected at all varities. Wendehenne *et al.*, (1998) and Nafie and Mazen, (2008) reported CAT plays a significant part in confiscating H_2O_2 from plant materials. A decrease in Catalase activity was prominent in healthy plants after pathogen inoculation which is related with amplified H_2O_2 level in stressed plant. Following the research of Melgar *et al.*, (2006) and Bestwick *et al.*, (1998), it was depicted that PODs are imperative constituent of plant stress responses which used to up or down regulate H_2O_2 amount in plant tissues. High POD level was allied with resistance in soybean against *Phytophthora sojae*. Singh *et al.*, (2011) enquiry is strongly related to current results in which he studied different biochemical compounds and reported that antioxidants (SOD, POD, CAT, Protein) concentration was found enhanced in inoculated leaves showing disease symptoms. Similarly, level of APX was downregulated with increasing intensity of infection. SOD enzyme was found a first line of defense against ROS and quick introduction of SOD helped to identify the pathogen's a-virulence factors. Following current research findings are reporting the enhancing level of SOD in injected leaves of resistant varieties and such results are strongly favored by Lebeda *et al.,* (2001); Hameed and Iqbal, (2014) as their findings are revealing augmented SOD activity. Its production in highly susceptible varieties was very high because of higher production of H_2O_2 which is toxic for plant. Asif *et al.,* (2018) conducted experiments on ROS regulating enzymes like catalases (CAT), phenolic, and peroxidases (POD), super oxide dismutase (SOD), Hydrogen peroxide Phenolics and protein. It was demonstrated the increase in activity of following enzymes in diseased induced plants of resistant variety. In resistant cultivars i.e Faisal Canola activity of SOD, POD, CAT, phenolic and H_2O_2 increased while protein activity was found (3.14 to 1.416mg/g of leaves). A very rapid increase in above mentioned parameters was observed in susceptible variety (Toria Selection A). Lamb and Dixon, (1997) exhibited that the level of H_2O_2 is a common product and its synthesis taken place in limited time frame after the actions of pathogen infection which caused it to delay during disease development. This is called H_2O_2 transient stage of early pathogen establishment. Such temporary level of H_2O_2 is measured as rapid plant action against pathogen occurrence and mainly allied with cell wall firmness that happens due to crosslinking responses. Meanwhile this enzyme can be working as a secondary messenger which carry signals for activation of defensive genes. In addition, it might be posing direct threat as toxic to any kind of pathogens. In inoculated plants the H_2O_2 assembled during transient establishment was instantly reduced at later on mediating as effective antioxidative system which saves plant to damage. Though, in uninoculated plants enhanced level of H_2O_2 was overdue and persisted at higher concentrations even at later time frame which caused host cell death due to more lipids peroxidation and thus indirectly paving way for the establishment of *A. brassicae*, a necrotrophic pathogen.

The capability of plants to overwhelmed oxidative stress comparatively count on initiation of SOD activity and afterward on the increasing level of down regulating ROS enzymes (Alscher *et al.,* 2002). According to this fact that SOD processing is known to be substrate inducible (Tsang *et al.,* 1991), an upsurge in SOD level associated to the augmented production of active oxygen species as substrate that lead to enhanced expression of genes encoding SOD. Our results are reliable with reported research of increased SOD activity in response to pathogen stress in sunflower (Gunes *et al.*, 2008). Therefore, it is prime important that hydrogen peroxide should instantly be condensed via antioxidant system into oxygen and water (Guo *et al.,* 2006). If the level of SOD and POD is overexpressed conjointly then scavenging mechanisms like CAT and POD enzyme

activities regarded as an important antidrought mechanism to cope with oxidative stress during water deficit conditions (McKersie *et al.,* 1999). Our results indicated a substantial increase in POD level in oilseed rape plants. Some previous studies, as parallel with our results, reported the increased POD activity in various plants, like sunflower (Gunes *et al.*, 2008), brassica species (Das and Uprety, 2006), (*Brassica juncia* (Asif *et al.,* 2018). Such findings expressed that, SOD and POD generally depicts concurrent induction and decrease associated with their co-regulation (Shigeoka *et al.,* 2002). The important point here is a decrease in POD activity which may reflect the low ROS scavenging capacity and increased damage in these cultivars under this condition. In terms of our results, CAT activity decreased in all experimental plant cultivars. The decline in CAT activity is regarded as a general response to many stresses (Herbinger *et al.,* 2002; Bakalova *et al.,* 2004; Jung, 2004; Pan *et al.,* 2006; Liu *et al.,* 2008). The reduction of CAT activity is due to the inhibition of enzyme synthesis or change in the assembly of enzyme subunits under stress conditions. Another phenomenon related to excessive production of Reactive Oxygen radicals is to initiate hypersensitive response (HR) via oxidative burst. HR is rapid activator of defense machinery of plant which limits water and nutrients of pathogen (Jones and Dangl, 2006; Glazebrook, 2005) and pathogen releases virulence factors which are recognized by the plant after that process. Such enzymes concludingly work for plant and defend from any damage and their study needs further exploration for genetically resistant plants development.

Conclusion

Enhanced biochemical contents like CAT, POD, SOD, Phenolic and H_2O_2 but decreased in protein content as compared to uninoculated leaves was observed. Such studies can be further incorporated in breeding program to identify and develop resistant source. These changes can be used as effective disease controlling mechanism and resistance inducer.

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