Available Online at ESci Journals
International Journal of Phytopathology
ISSN: 2305-106X (Online), 2306-1650 (Print)
http://www.escijournals.net/phytopathology

A PIVOTAL ROLE OF REACTIVE OXYGEN SPECIES IN NON-HOST RESISTANCE MECHANISMS IN LEGUME AND CEREAL PLANTS TO THE INCOMPATIBLE PATHOGENS

Yaser M. Hafez
Department of Agricultural Botany, (Plant Pathology Section) Faculty of Agriculture, Kafr-El-Sheikh University, 33516 Kafr-El-Sheikh, Egypt.

A B S T R A C T

Most of plants under normal conditions are resistant to most of the incompatible pathogens (viral, fungal and bacterial infections). This is called ″non-host resistance (NHR) phenomenon″. Till now it is not clear the non-host resistance mechanisms. As a result of inoculation of legume (pea and soybean) and cereal (barley and wheat) plants with compatible and incompatible pathogens, strong resistance symptoms were observed in the non-host/incompatible pathogen combinations as compared with host/compatible pathogen combinations which showed severe infection (susceptibility). Levels of reactive oxygen species (ROS) mainly hydrogen peroxide (H_2O_2) and superoxide (O_2.-) were significantly increased early 6, 12, 24 and 36 hours after inoculation (hai) in the non-host plants as compared with host plants. Interestingly enough that the activities of the antioxidant enzymes such as catalase (CAT), dehydroascorbate reductase (DHAR) and peroxidase (POX) were not significantly increased at the same early time 6 - 36 hai in the non-host plants. However, these enzymes were significantly increased later on 48, 72 and 96 dai in the non-host plants as compared with host plants. It seems that early accumulation of H_2O_2 and O_2.- could have a dual roles, first role is inhibiting or killing the pathogens early in the non-host plants, second immunization of the non-host plants by stimulating the activities of the antioxidant enzymes later on which thereby, neutralize the harmful effect of ROS and consequently suppressing disease symptoms. The author recommends giving more attention to these new mechanisms of non-host resistance particularly in relation to ROS levels and antioxidant activities which are very important for plant breeders and useful for finding alternative control strategies as well.

Keywords: Antioxidants, ROS, Non-host resistance, legumes, cereals.

INTRODUCTION

Non-host resistance (NHR) is a resistance showed by an entire plant species to all genetic variants of a non-adapted pathogen species. NHR refers to plant species immunity against the majority of microbial pathogens and represents the most healthy and strong form of plant resistance in nature (Yulin *et al.,* 2012; Lipka *et al.,* 2010). NHR phenomenon explains why most of plants are immune to the majority of pathogens and normally healthy. Mechanisms supporting NHR remain relatively unknown compared with the well-studied host resistance mediated by the products of plant resistance (R) genes, which establish pathogen race- or plant cultivar-specific resistance (Schulze-Lefert and Bieri 2005; Dangl and Jones 2001). NHR plants to pathogens mainly fungal, bacterial and viral infections can be defined as an innate non-specific resistance which is effective against all known isolates of several species of the pathogens (Király *et al.,* 2007; Thordal-Christensen, 2003). This NHR resistance is a strong and very effective type of plant immunity (Heath, 2000). On the other hand, appropriate pathogens escape defense reactions of the host by avoiding recognition or suppressing resistance of non-host or host but resistant plants (Schulze-Lefert and Panstruga, 2003). Researchers showed some experiments in relation to genetics of non-host type of resistance. However, only a few biochemical results are available as regards the formation of host cell wall appositions (papillae), local accumulation of autofluorogens and reactive oxygen species (ROS), such as

* Corresponding Author:
Email: hafezyasser@gmail.com

hydrogen peroxide (H_2O_2) (Trujillo *et al.,* 2004; Hückelhoven *et al.,* 2001; Carver *et al.,* 1992).

Pea (*Pisum sativum* L.) is one of the most important legume vegetable crops grown in Egypt and many other countries all over the world. It has many nutritional values such as high content of protein, carbohydrates, phosphorus, iron, calcium and vitamins A and B (Watt and Merrill, 1963). Soybean (*Glycine max* L.) is one of the world's most important sources of oil and protein. It has the highest protein content among leguminous crops (Abdel-Monaim *et al.,* 2011). Barley (*Hordeum vulgar*e L.) ranks the fourth among the major cereal grians in terms of World and Egyptian production after maize, wheat and rice crops (Hafez *et al.,* 2014). Wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the world for both human food and animal feed (Abdelaal *et al.,* 2014; Chen *et al*., 2003). These important plants can be seriously damaged by *Botrytis cinerea*, *Alternaria solani*, *Blumeria graminis* f.sp. *hordei* (*Bgh*) and *Blumeria graminis* f.sp. *tritici* (*Bgt*). Due to the great and economic damages of these host/pathogen interactions, research into the functional and characterization of new resistance mechanisms are required. According to our knowledge, not too much experimental results have been achieved which would explain the question: what is arresting or killing the pathogens in the non-host resistant plants? However, some promising and preliminary results were obtained which indicated that ROS have a pivotal role in the arrest of pathogens in non-host plants. However, till now little is known about the nature of effective defense mechanisms in pea, soybean, barley and wheat plants to incompatible pathogens, especially pathogens with economic and biological importance.

The aim of this research study was to characterize the mode of action of the non-host resistance mechanisms in pea, soybean, barley and wheat to the *Papya ring spot virus* (*PRSV*), *B. cinerea*, *B. graminis* f. sp. *riticit* and *B. graminis* f. sp. *h*ordei respectively at the morphological, histological and biochemical levels which thereby, very useful for plant breeders and sustainable crop protection.

MATERIALS AND METHODS

Plant Materials: Pea (*Pisum sativum* L.) cultivar (cv.) Little Marvel and soybean (*Glycine max* L.) cv. Giza 111 seeds were obtained from Food Legumes Research Section, Sakha, Kafr El-Sheikh, Field Crops Research Institute (FCRI), Agricultural Research Station (ARC), Egypt. Barley (*Hordeum vulgar*e L.) cv. Giza 123 seeds obtained from Dept. of Barley, FCRI, ARC, Egypt. Wheat (*Triticum aestivum* L.) cv. Sakha 61 was obtained from Wheat Pathology Department, Sakha, Kafr El-Sheikh, ARC, Egypt. Seeds were sown in a 12-cm plastic pots containing soil mixed with peat moss (1:1) and grown in the greenhouse. Temperature was 18-23 ^{o}C, with 16 hours photoperiod per day using supplemental light with a light intensity of 160 µE m^{-2} s^{-1} and relative humidity 75-80%. These experiments were conducted in the laboratories, green houses and growth chambers of Botany Department, Faculty of Agriculture, Kafr-Elsheikh University, Egypt as well as Plant Pathophysiology Department, Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary during the years 2012 -2014.

Plant Pathogens: The *Papaya ringspot virus* (PRSV) Egyptian isolate was obtained from Plant Pathology and Biotechnology Laboratory, Faculty of Agriculture, Kafr-Elsheikh University, Egypt. PRSV was maintained on the host susceptible squash (*Cucurbita pepo*). For mechanical virus inoculation, viral-infected leaves were homogenized in tap water. Carborundum was used as an abrasive for virus and mock inoculations.

Botrytis cinerea Pers., Bc-1 and *Alternaria solani* isolates were kindly supplied by Prof. László Vajna, Plant Protection Institute, Hungarian Academy of Sciences, Hungary. These pathogens were maintained on potato dextrose agar medium (PDA) under continuous fluorescent light. For inoculation, agar discs 5 mm in diameter were cut from 7-day-old cultures of the fungus *Botrytis cinerea* and 15-days old culture of the fungus *Alternaria solani* then, placed on the surface of pea and soybean leaves, respectively. Leaves were cut and put on wet filter paper in a Petri dish and held at 20°C in continuous light for at least 3 days for pea and 2-5 days for soybean (Hafez *et al.,* 2012).

Barley powdery mildew (*Blumeria graminis* f. sp. *hordei*) and wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*) Egyptian specimens were maintained under greenhouse conditions and were used for all inoculation experiments. Powdery mildew inocula were dispersed in the greenhouse atmosphere by placing plants of barley and wheat bearing sporulating colonies of both pathogens beneath ventilation fans of the greenhouse (Hafez and Kiraly, 2003).

Disease Severity Assessments: Disease severity percentage (%) of pea inoculated with *B. cinerea* (host)

and PRSV (non-host) as wel as soybean inoculated with *A. solani* (host) and *B. cinerea* (non-host) were measured as lesion diameter (cm^2) according to Hafez *et al.,* 2004. However, the disease severity percentage (%) of barley inoculated with barely powdery mildew (host) and inoculated with wheat powdery mildew (non-host) as well as wheat inoculated with wheat powdery mildew (host) and inoculated with barley powdery mildew (no-host) were determined as follows: Ten plants of each replicate were scored visually for percentage of leaf area covered by powdery mildew on a 0 (resistant) to 10 (susceptible) scale three times 3, 6 and 9 days after inoculation (dai) in each experiment. For analysis, disease scores were converted using the modified logarithmic scale of Horsfall–Barrett (Horsfall and Cowling, 1978) and Hafez et al., 2014. The scale was 0 = 0 %, 1 = 0–3 %, 2 = +3–6 %, 3 = +6–12 %, 4 = +12–25 %, 5 = +25–50 %, 6 = +50–75 %, 7 = +75–88 %, 8 = +88–94 %, 9 = +94–97 % and 10 = +97–100 %. Disease severity index (DSI) was calculated according to Kim *et al*., 2000 using the following formula:

$$\text{DSI} = \frac{\sum \text{R atings of each plant}}{10 \times \text{Number of plants rated}}$$

Histochemical Analysis of ROS: Histochemical staining for $O_2^{\cdot-}$ production in leaf tissue was based on the ability of $O_2^{\cdot-}$ to reduce nitro blue tetrazolium (NBT). $O_2^{\cdot-}$ was visualised as a purple coloration of NBT. Leaf discs (2 cm) of pea and soybean plants as well as hall barley and wheat leaves were vacuum infiltrated or injected (Hagborg, 1970) with 10 mM potassium phosphate buffer (pH 7.8) containing 0.1 w/v % NBT (Sigma–Aldrich, Germany) according to Ádám *et al.,* (1989). NBT-treated samples were incubated under daylight for 20 min and subsequently cleared in 0.15 % trichloroacetic acid (wt/vol) in ethanol: chloroform 4:1 (vol/vol). The solution was exchanged once during the next 48 h of incubation (Hückelhoven *et al.,* 1999). Subsequently, leaf discs and leaves were stored in 50% glycerol for evaluation.

For histochemical analysis of hydrogen peroxide (H_2O_2), leaf discs and hall leaves were infiltrated with 0.1% 3, 3-diaminobenzidine (DAB) in 10 mM Tris buffer (pH 7.8). Samples were incubated under daylight for two hours after the vacuum infiltration. Following staining, leaves were cleared as described above and the intensity of brown color was estimated (Hückelhoven *et al.,* 1999). Levels of $O_2^{\cdot-}$ and H_2O_2 were estimated 6, 12, 24, 36, and 48 hours after inoculation. These tests were repeated five times during six independent experiments.

Biochemical Assays of Antioxidant Enzymes: The tested antioxidant enzyme activities were measured on 5 weeks-old pea and soybean plants as well as 2 weeks-old barley and wheat plants. For enzyme assays in plants, 0.5 g detached leaves material from 5 and 2 weeks old plants, respectively, was homogenized at 0-4˚C in 3 ml of 50 mM TRIS buffer (pH 7.8), containing 1 mM EDTA-Na_2 and 7.5% polyvinylpyrrolidone 1, 2, 3 and 4 days after inoculation. The homogenates were centrifuged (12,000 rpm, 20 min, 4˚C), and the total soluble enzyme activities were measured spectrophotometrically in the supernatant (Hafez, 2010, Hafez et al., 2014). All measurements were carried out at 25˚C, using the model UV-160A spectrophotometer (Shimadzu, Japan).

Activity of catalase (CAT) was determined spectrophotometrically according to Aebi (1984). Decomposition of H_2O_2 by catalase results in the decrease of the ultraviolet absorption of hydrogen peroxide at 240 nm. Enzyme activity can be calculated from this decrease. The reaction mixture contained, in a final volume of 2.15 ml, 2 ml 0.1 M Na-phosphate buffer (pH 6.5), 100 µl hydrogen peroxide and 50 µl leaf extract supernatant. The solution is mixed, and then the absorption change is registered for 3 min at 240 nm using a quartz cuvette.

Activity of dehydroascorbate reductase (DHAR) was determined spectrophotometrically according to Asada (1984). The reaction mixture contained, in a final volume of 2.3 ml, 50 mM sodium phosphate buffer (pH 6.5), 0.5 mM dehydroascorbate (DHA), 1.0 mM reduced glutathione (GSH), 0.1 mM EDTA and 0.1 ml supernatant. The assay was carried out in quartz cuvettes following the increase in absorbance at 265 nm due to the formation of ascorbate with extinction coefficient 14 mM^{-1} cm^{-1} (Klapheck *et al*., 1990). The reaction rate was corrected for the non-enzymatic reduction of dehydroascorbate by GSH.

Activity of peroxidase (POX) was directly determined of the crude enzyme extract according to a typical procedure proposed by Hammerschmidt *et al.,* (1982). Changes in absorbance at 470 nm were recorded every 30 sec intervals for 3min. Enzyme activity was expressed as increase in absorbance min^{-1} g^{-1} fresh weight.

Statistical Analysis: Six experiments were conducted in a completely randomized design with five replicates for each treatment. Data represent the mean ± SD. Student's t-test was used to determine

whether significant difference (P<0.05) existed between mean values according to O'Mahony (1986).

RESULTS AND DISCUSSION

Disease Severity and Disease Symptoms of Host and Non-host/Pathogen Combinations: In the "non-host" plants pea, soybean, barley and wheat showed resistance against *Papaya ringspot virus*, *B. cinerea*, *Blumeria graminis* f. sp. *tritici* and *Blumeria graminis* f. sp. *hordei*, respectively compared to the "host" plants pea, soybean, barley and wheat inoculated with *Botrytis cinerea*, *Alternaria solani*, *Blumeria graminis* f. sp. *hordei* and *Blumeria graminis* f. sp. *tritici*, respectively showed strong susceptibility. Disease severity percentage was significantly severe strongly in all the host/pathogen combinations as compared to the non-host/pathogen combinations (Figure 1 and Table 1).

Table 1. Reaction of host and non-host/pathogen combinations.

Plants	Host	Results	Non-host	Results
Pea	*Botrytis cinerea*	S	*Papaya ringspot virus*	R
Soybean	*Alternaria solani*	S	*Botrytis cinerea*	R
Barley	*Blumeria graminis* f. sp. *hordei*	S	*Blumeria graminis* f. sp. *tritici*	R
Wheat	*Blumeria graminis* f. sp. *tritici*	S	*Blumeria graminis* f. sp. *hordei*	R

S= susceptible, R= resistant

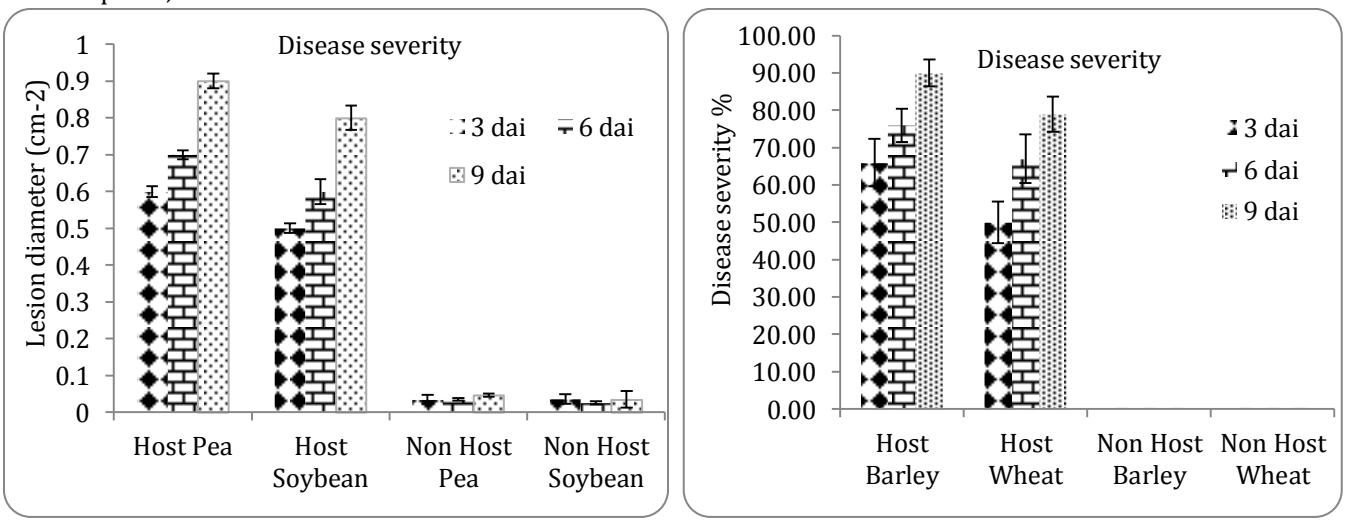

Figure 1. Disease severity percentage (%) of host and non-host/pathogen combinations 3, 6 and 9 days after inoculation (dai) of pea, soybean, barley and wheat plants. Host Pea: leaves inoculated with *Botrytis cinerea*. Host soybean: leaves inoculated with *Alternaria solani*. Non Host Pea: leaves inoculated with Papaya ringspot virus. Non Host soybean: leaves inoculated with *B. cinerea*. Host Barley: leaves inoculated with *Blumeria graminis f. sp. hordei* (Bgh). Non Host Barley: leaves inoculated with *Blumeria graminis f. sp. tritici* (Bgt). Host Wheat: leaves inoculated with Bgt. Non Host Wheat: leaves inoculated with Bgh.

Disease symptoms were also significantly appeared and visible in the host/pathogen combinations compared to the non-host which no symptoms appeared (Figure 2). Similar results have been obtained by Fabro *et al.* (2011) in which found that in the "non-host" plant *Brassica rapa* (turnip) which was more effectors of *Hyaloperonospora arabidopsidis* are recognized than in *Arabidopsis thaliana* which is a "host" of this oomycete pathogen. This could be a possible cause of the inability of *H. arabidopsidis* to grow in turnip.

In other words, the host plant cannot recognize a subset of effectors of its own pathogen which are recognized, and therefore induce an immune reaction in the non-host. However, it is still an unanswered question, how this immune reaction can inhibit pathogens in non-host plants? (Kiraly *et al.,* 2013).

Levels of Reactive Oxygen Species in Host and Non-host/Pathogen Combinations: Purple discoloration of superoxide ($O_2^{\cdot-}$) and brown discoloration of hydrogen peroxide (H_2O_2) reflect the intensivity of ROS levels in the leaves which cleared from chlorophyll (Figure 2). Levels of ROS mainly $O_2^{\cdot-}$ and H_2O_2 significantly accumulated early 6, 12, 24 and 36 hours after inoculation (hai) in all non-host/pathogen combinations in pea, soybean, barley and wheat plants (Figure 3, 4 and 5).

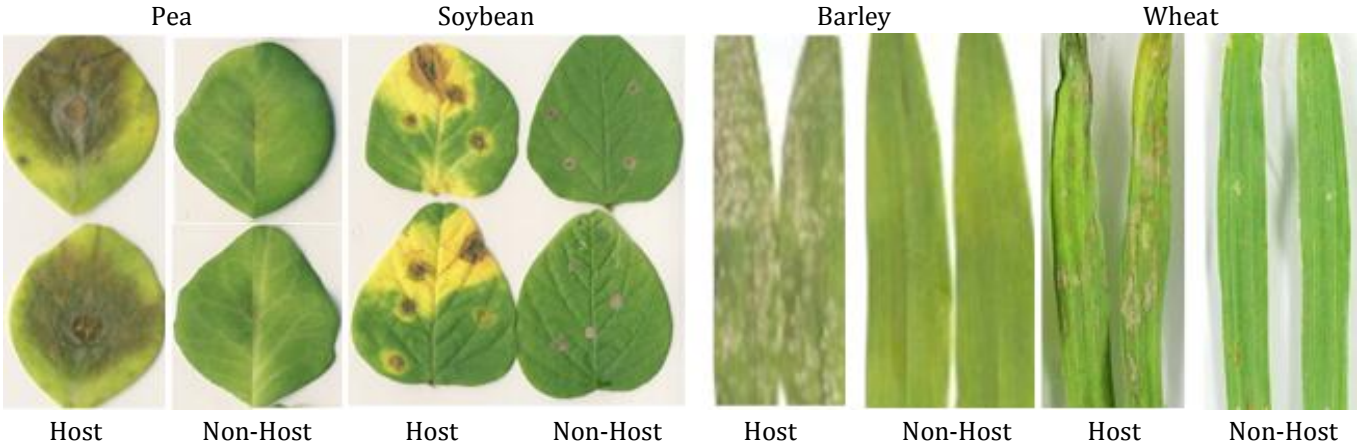

Figure 2. Disease symptoms of host and non-host/pathogen combinations 4 days after inoculation (dai) in pea and soybean as well as 2 dai in barley and wheat plants. Host Pea: leaves inoculated with *Botrytis cinerea*. Host soybean: leaves inoculated with *Alternaria solani.* Non Host Pea: leaves inoculated with *Papaya ringspot virus*. Non Host soybean: leaves inoculated with *B. cinerea*. Host Barley: leaves inoculated with *Blumeria graminis* f. sp. *hordei (Bgh).* Non Host Barley: leaves inoculated with *Blumeria graminis* f. sp. *Tritici* (*Bgt*). Host Wheat: leaves inoculated with *Bgt.* Non Host Wheat: leaves inoculated with *Bgh.*

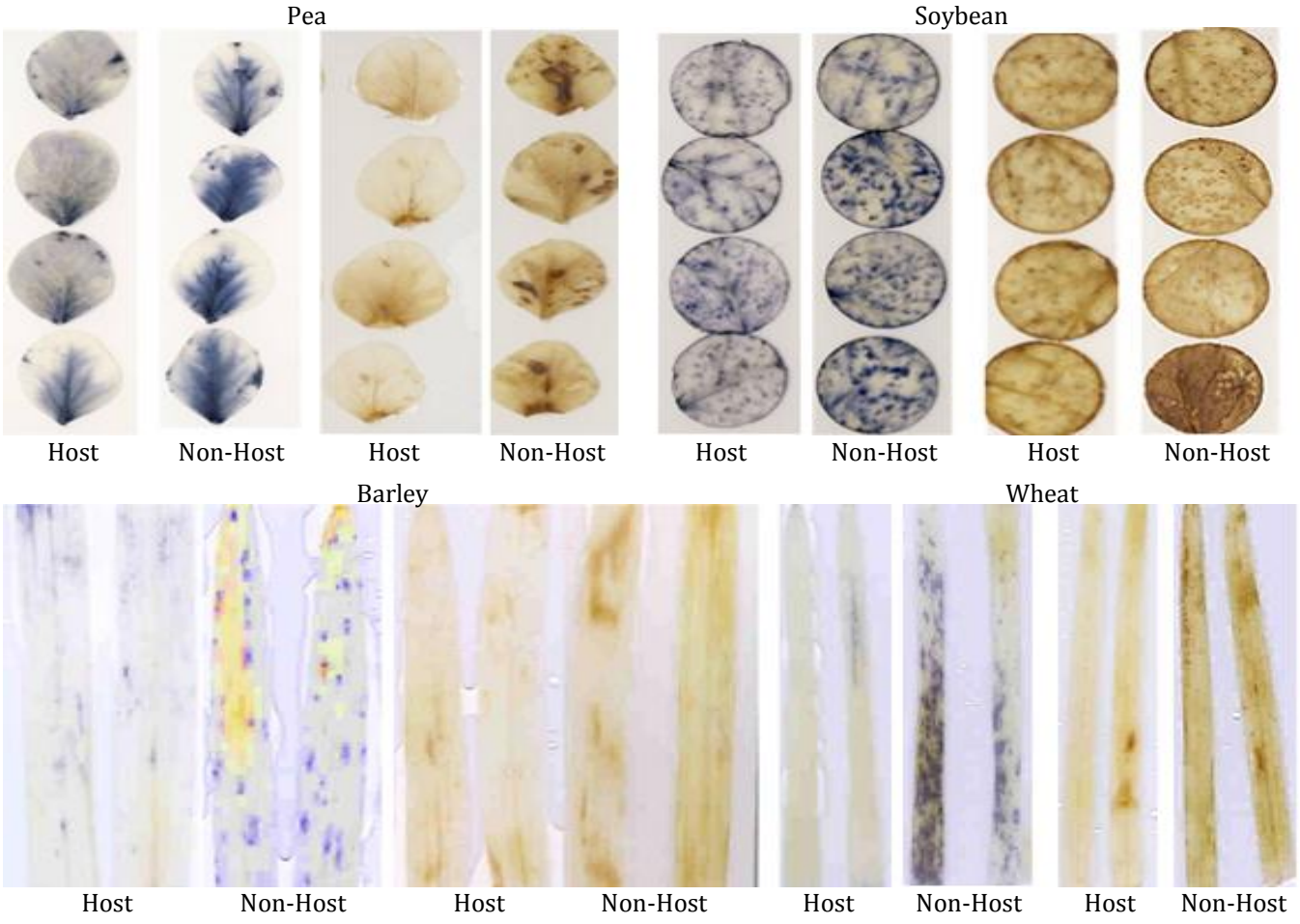

Figure 3. Purple discoloration of superoxide ($O_2^{\cdot-}$) and brown discoloration of hydrogen peroxide (H_2O_2) of host and non-host/pathogen combinations 12 hours after inoculation (dai) in pea and soybean as well as 6 hai in barley and wheat plants. Host Pea: leaves inoculated with *Botrytis cinerea*. Host soybean: leaves inoculated with *Alternaria solani.* Non Host Pea: leaves inoculated with *Papaya ringspot virus*. Non Host soybean: leaves inoculated with *B. cinerea*. Host Barley: leaves inoculated with *Blumeria graminis* f. sp. *hordei (Bgh).* Non Host Barley: leaves inoculated with *Blumeria graminis* f. sp. *Tritici* (*Bgt*). Host Wheat: leaves inoculated with *Bgt.* Non Host Wheat: leaves inoculated with *Bgh.*

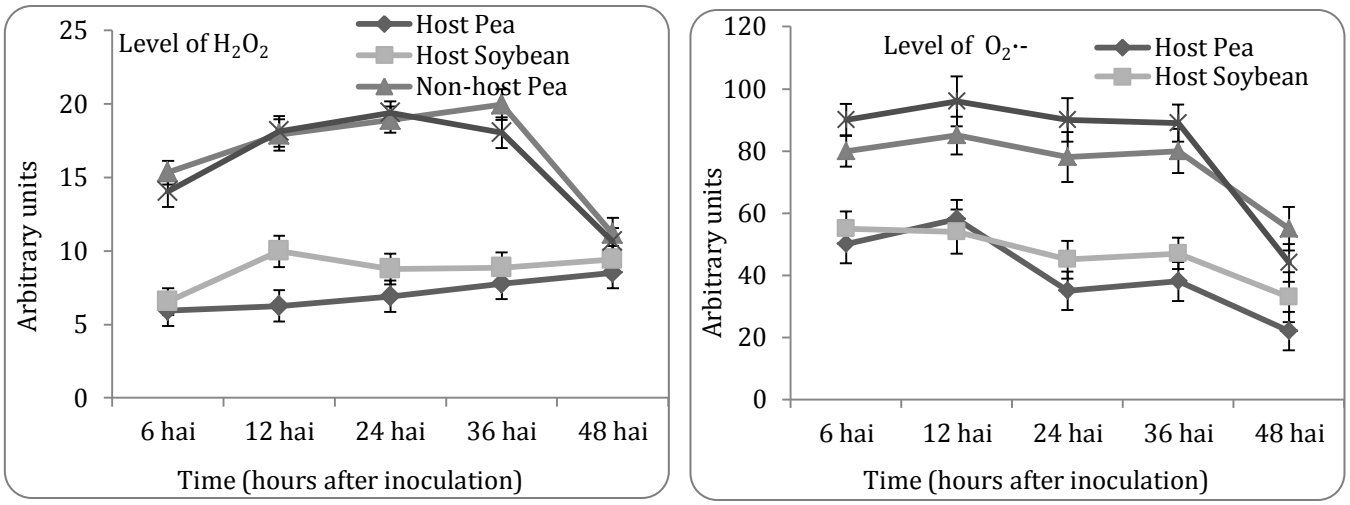

Figure 4. Levels of hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\cdot-}$) of host and non-host/pathogen combinations 6, 12, 24, 36 and 48 hours after inoculation (hai) in pea and soybean plants. Host Pea: leaves inoculated with *Botrytis cinerea*. Host soybean: leaves inoculated with *Alternaria solani.* Non Host Pea: leaves inoculated with *Papaya ringspot virus*. Non Host soybean: leaves inoculated with *B. cinerea*. Host Barley: leaves inoculated with *Blumeria graminis* f. sp. *hordei (Bgh).* Non Host Barley: leaves inoculated with *Blumeria graminis* f. sp. *Tritici* (*Bgt*). Host Wheat: leaves inoculated with *Bgt.* Non Host Wheat: leaves inoculated with *Bgh.*

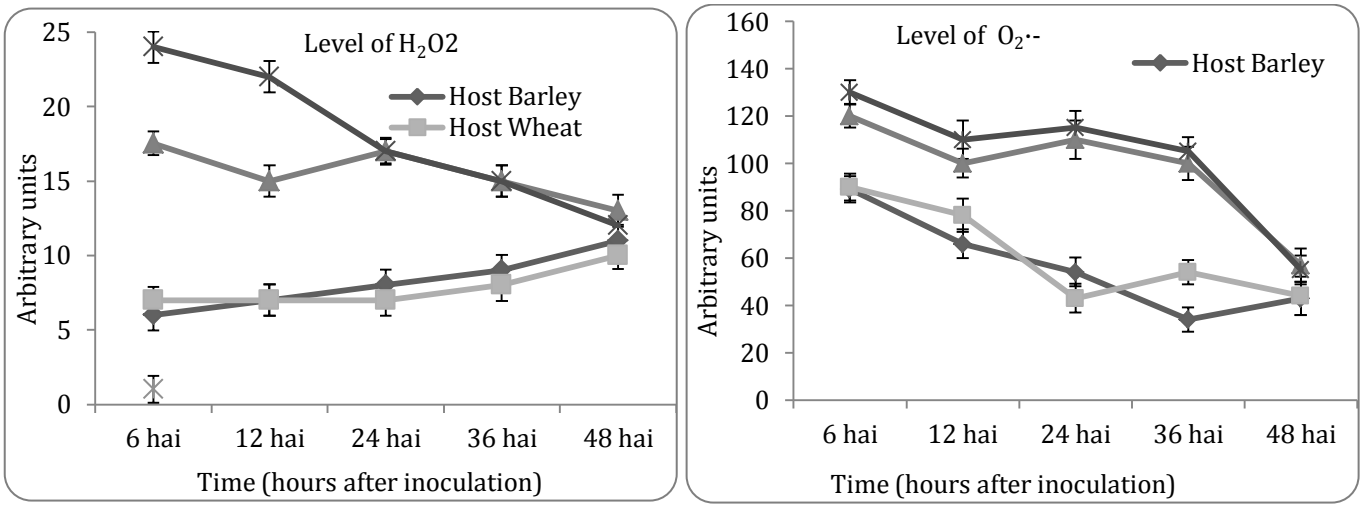

Figure 5. Levels of hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\cdot-}$) of host and non-host/pathogen combinations 6, 12, 24, 36 and 48 hours after inoculation (hai) in barley and wheat plants. Host Pea: leaves inoculated with *Botrytis cinerea*. Host soybean: leaves inoculated with *Alternaria solani.* Non Host Pea: leaves inoculated with *Papaya ringspot virus*. Non Host soybean: leaves inoculated with *B. cinerea*. Host Barley: leaves inoculated with *Blumeria graminis* f. sp. *hordei (Bgh).* Non Host Barley: leaves inoculated with *Blumeria graminis* f. sp. *Tritici* (*Bgt*). Host Wheat: leaves inoculated with *Bgt.* Non Host Wheat: leaves inoculated with *Bgh.*

Activities of Antioxidant Enzymes in Host and Non-host/Pathogen Combinations: Activities of antioxidant enzymes such as catalase (CAT), dehydroascorbate reductase (DHAR) and peroxidase (POX) were not changed or even increased early 6, 12, 24 and 36 hours after inoculation (hai) in all non-host/pathogen combinations in pea, soybean, barley and wheat plants, however, these enzymes were increased significantly a little bit later at 48, 72 and 96 hai (Figure 6 and 7).

One can say that in these non-host resistant pea, soybean, barley and wheat plants the early accumulation of $O_2^{\cdot-}$ and H_2O_2 not only inhibit or kill the incompatible pathogens 6-36 hai but also stimulate the activities of antioxidants CAT, DHAR and POX alter on 24-96 hai. Our results are supported by our previous results in which pointed out that under natural conditions the up-regulation of antioxidant defense systems seems to be a general response to oxidative stress (Hafez *et al.,* 2012).

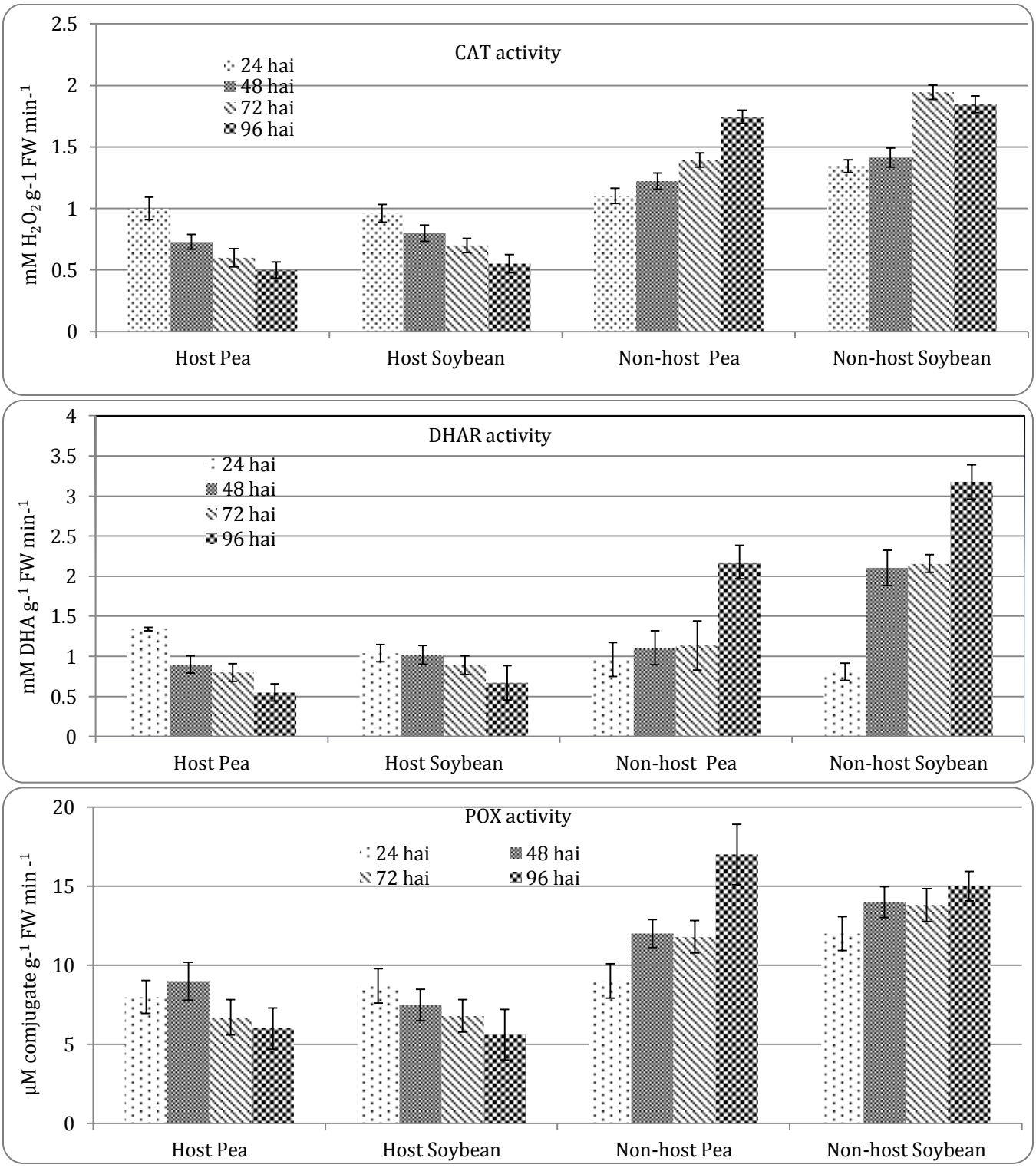

Figure 6. Activities of antioxidant enzymes catalase (CAT), dehydroascorbate reductase (DHAR) and peroxidase (POX) in host and non-host/pathogen combinations 24 48, 72 and 96 hours after inoculation (hai) in pea and soybean plants. Host Pea: leaves inoculated with *Botrytis cinerea*. Host soybean: leaves inoculated with *Alternaria solani.* Non Host Pea: leaves inoculated with *Papaya ringspot virus*. Non Host soybean: leaves inoculated with *B. cinerea*. Host Barley: leaves inoculated with *Blumeria graminis* f. sp. *hordei (Bgh).* Non Host Barley: leaves inoculated with *Blumeria graminis* f. sp. *Tritici* (*Bgt*). Host Wheat: leaves inoculated with *Bgt.* Non Host Wheat: leaves inoculated with *Bgh.*

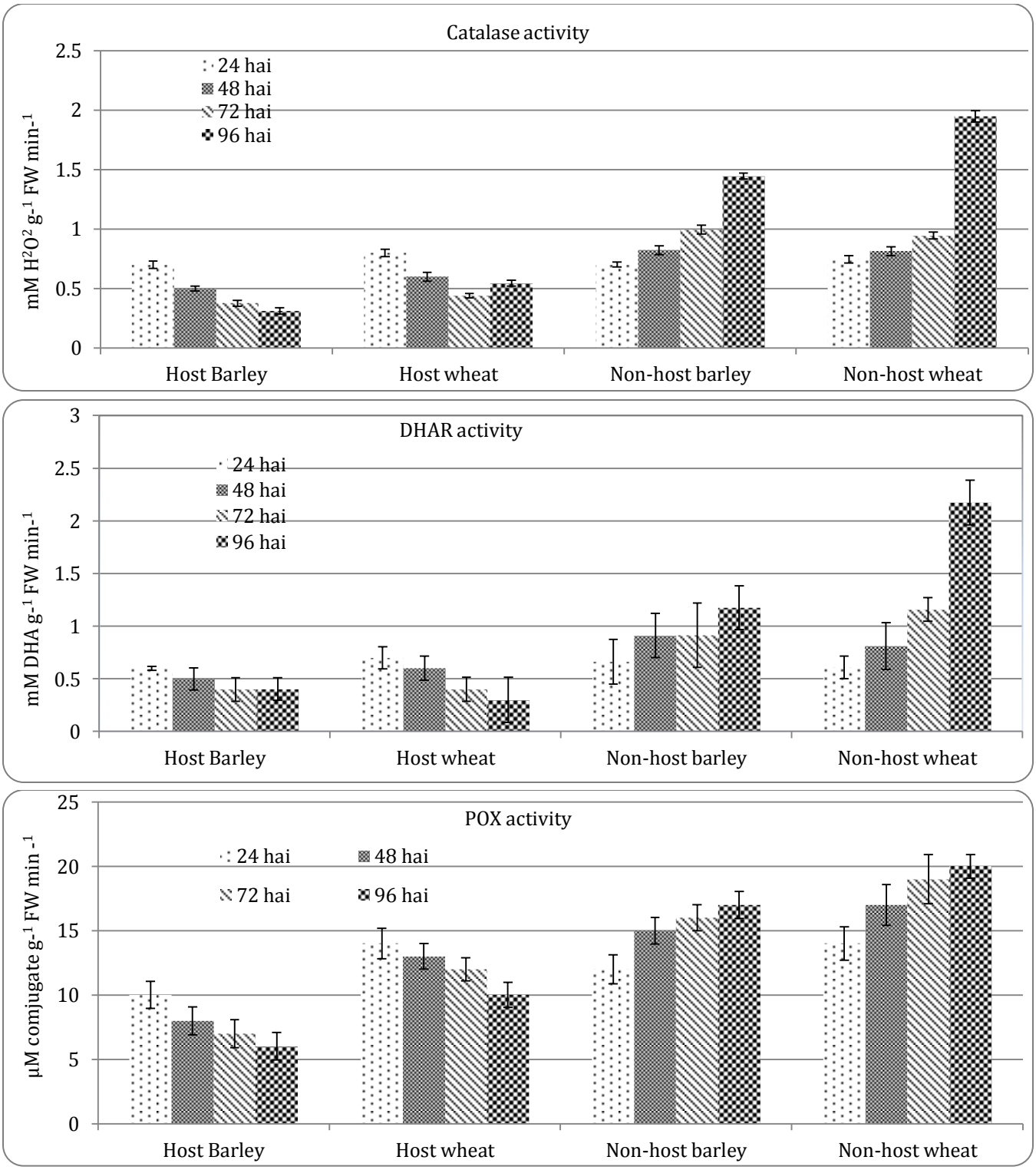

Figure 7. Activities of antioxidant enzymes catalase (CAT), dehydroascorbate reductase (DHAR) and peroxidase (POX) in host and non-host/pathogen combinations 24 48, 72 and 96 hours after inoculation (hai) in barley and wheat plants. Host Pea: leaves inoculated with *Botrytis cinerea*. Host soybean: leaves inoculated with *Alternaria solani.* Non Host Pea: leaves inoculated with *Papaya ringspot virus*. Non Host soybean: leaves inoculated with *B. cinerea*. Host Barley: leaves inoculated with *Blumeria graminis* f. sp. *hordei (Bgh).* Non Host Barley: leaves inoculated with *Blumeria graminis* f. sp. *Tritici* (*Bgt*). Host Wheat: leaves inoculated with *Bgt.* Non Host Wheat: leaves inoculated with *Bgh.*

The early accumulation of ROS in the non-host resistant plants up-regulate the antioxidants go along with the results which proved that H_2O_2 as one of the most important reactive oxygen species associated with oxidative stress, can up-regulate antioxidant systems even at very low concentrations against abiotic stress (Gechev *et al.,* 2002) and also immunize plants, therefore, induces resistance to symptom development by suppressing pathogen-induced host cell and tissue necroses but not pathogen multiplication, while enhancing activities of at least three antioxidant enzymes such as catalase, guaiacol peroxidase and ascorbate peroxidase (Hafez *et al.,* 2012). It was pointed out that often the only evidence that oxidative stress has occurred in vivo may be it is the cause of up-regulation of antioxidant defense systems (Halliwell and Gutteridge, 1999). The high activities of enzymatic and nonenzymatic antioxidants neutralized the harmful effects of ROS (oxidative stress). Particularly, H_2O_2 seems to play a dual role by eliciting localized death of plant and pathogen cells and as a diffusible signal for the induction of antioxidant and pathogenesis-related genes in adjacent plant tissues (Hafez *et al.,* 2012; Wu *et al.,* 1997; Levine *et al.,* 1994).

It worth mentioning that the author use for this research four model important plants, two from legumes (pea and soybean) and two from cereals (barley and wheat) which support his new findings. It can be concluded that new histochemical and biochemical mechanisms of non-host resistance mechanisms were found by the author in this research article. ROS mainly superoxide and hydrogen peroxide accumulated early after inoculation which may be killing or inhibiting the pathogens in these non-host plants. This early accumulation of ROS stimulated the antioxidant enzymes activities later on which thereby could immunize plants by suppressing disease symptoms and neutralize the harmful effect of ROS against these incompatible pathogens. It is recommended to the researchers and plant breeders to give more attention to these interesting new results to find new strategies for future integrated control pest management practices and sustainable crop protection.

AKNOWLEDGEMENT

This research was conducted and supported by the Plant Pathology and Biotechnology Lab., (under accreditation of ISO/17025), EPCRS Excellence Centre, Dept. of Agric. Botany, Fac. of Agric., Kafrelsheikh University, Kafr-Elsheikh, Egypt and Plant Protection Institute, Hungarian Academy of sciences, Hungary during the years 2012-2014. The EPCRS Excellence Centre Project is funded by Management Supporting Excellence (MSE), Development Projects Management Unit, Ministry of Higher Education, Egypt.

REFERENCES

Abdelaal, K. A. A., Y. M. Hafez, M. Adel Samar, W. A. Youseef and M. M. Badr. 2014. Biochemical, histological and molecular changes in some Egyptian wheat varieties infected with stripe rust (*Puccinia striiformis* f.sp. *tritici*). Egyptian J. Biol.Pest Cont., 24 (2): 421-429.

Abdel-Monaim M. F., M. E. Ismail and K. M. Morsy. 2011. Induction of systematic resistance in soybean plants against *Fusarium* wilt disease by seed treatment with benzothiadiazole and humic acid. Not. Sci. Biol., 3(2):80-89.

Ádám A., T. Farkas, G. Somlyai , M. Hevesi and Z. Király. 1989. Consequence of $O_{2^{\cdot-}}$ generation during a bacterially induced hypersensitive reaction in tobacco: deterioration of membrane lipids. Physiol. Molec. Plant Pathol., 34: 13-26.

Aebi, H. 1984. Catalase *in vitro*. Methods Enzymol., 105: 121-126.

Asada, K. 1984. Chloroplast formation of reactive oxigen and its scavenging. In: Colowick SP, Kaplan NO (eds.): Methods Enzymol,.Vol. 105. Acad. Press, New York, Pp.422-429.

Carver, T. L. W., R. J. Zeyen, M. P. Robbins and G. A. Dearne. 1992. Effects of the PAL inhibitor, AOPP, on oat, barley and wheat cell responses to appropriate and inappropriate formae speciales of *Erysiphe graminis* DC. Physiol. Mol. Plant Pathol., 41: 397-409.

Chen, X., A. S. Marcelo, Y. Guiping, S. Jun and J. Dubcovsky. 2003. Development of Sequence Tagged Site and Cleaved Amplified Polymorphic Sequence Markers for Wheat Stripe Rust Resistance Gene Yr5. Crop Sci. 43: 2058-2064.

Dangl, J. L. and J. D. G. Jones. 2001. Plant pathogens and integrated defense responses to infection. Nature, 411:826-833.

El-Zahaby, H. M., G. Gullner and Z. Király. 1995. Effects of powdery mildew infection of barley on the ascorbate-glutathione cycle and other antioxidants in different host-pathogen interactions. Phytopathol., 85: 1225-1230.

Fabro, G., J. Steinbrenner, M. Coates, N. Ishaque, L. Baxter, et al., 2011. Multiple candidate effectors from the oomycete pathogen *Hyaloperonospora arabidopsidis* suppress host plant immunity. PLoS Pathog.7,e1002348. doi:10.1371/journal.ppat.1002348.

Gechev, T., I. Gadjev, F. Van Breusegem, D. Inzé, S. Dukiandjiev, V. Toneva and I. Minkov. 2002. Hydrogen peroxide protects tobacco from oxidative stress by inducing a set of antioxidant enzymes. Cell Mol. Life Sci., 59:708-714.

Hafez, Y. M. and Z. Király. 2003. Role of hydrogen peroxide in symptom expression of barley susceptible and resistant to powdery mildew. Acta Phytopathol., Entomol., Hung., 38: 227-236.

Hafez, Y. M., J. Fodor and Z. Király. 2004. Establishment of systemic acquired resistance confers reduced levels of superoxide and hydrogen peroxide in TMV-infected tobacco leaves. Acta Phytopath. Entomol. Hung., 39: 347-359.

Hafez, Y. M. 2010. Control of *Botrytis cinerea* by the resistance riducers benzothiadiazole (BTH) and hydrogen peroxide on white pepper fruits under postharvest storage. Acta Phytopathol. Entomol. Hung., 45 (1): 13-29.

Hafez, Y. M., R. Bacsó, Z. Király, A. Künstler and L. Király .2012. Up-regulation of antioxidants in tobacco by low concentrations of H_2O_2 suppresses necrotic disease symptoms**.** Phytopathol., 102: 848-856.

Hafez, Y. M., R. Y. Mourad, M. Mansour and Kh. A. A. Abdelaal. 2014. Impact of non-traditional compounds and fungicides on physiological and biochemical characters of barely infected with *Blumeria graminis* f.sp. *hordei* under field condtition. Egyptian J. Biol. Pest Cont., 24 (2): 445-453.

Hagborg, W. A. F. 1970. A device for injecting solutions and suspensions into thin leaves of plants. Can. J. Bot., 48: 1135-1136.

Harrach, B. D, J. Fodor, M. Pogány, J. Preuss and B. Barna. 2008. Antioxidant, ethylene and membrane leakage responses to powdery mildew infection of near-isogenic barley lines with various types of resistance. Eur. J. Plant Pathol., 121: 21-33.

Halliwell, B. and J. M. C. Gutteridge. 1999. Free Radicals in Biology and Medicine. Oxford University Press, Inc., New York.

Hammerschmidt, R., E. M. Nuckles and J. Kuć. 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol. Plant Pathol., 20(1):73-82.

Heath, M. C. 2000. Nonhost resistance and nonspecific plant defenses. Curr. Opin. Plant Biol., 3: 315-319.

Horsfall, J. G. and E. B. Cowling. 1978. Pathometr: the measurement of plant disease. In: Horsfall, J.G. and E.B. Cowling, (Eds), Plant Disease: An Advanced Treatise. New York: Academic, Vol. II, pp. 120-136.

Hückelhoven, R., J. Fodor, C. Preis and K-H. Kogel. 1999. Hypersensitive cell death and papilla formation in barley attacked by the powdery mildew fungus are associated with H_2O_2 but not with salicylic acid accumulation. Plant Physiol., 119: 1251-1260.

Hückelhoven, R., C. Dechert and K-H. Kogel. 2001. Non-host resistance of barley is associated with a hydrogen peroxide burst at sites of attempted penetration by wheat powdery mildew fungus. Mol. Plant Pathol., 2: 199-205.

Kim, H. S., G. L. Hartman, J. B. Manandhar, G. L. Graef, J. R. Steadman and B. W. Dier. 2000. Reaction of soybean cultivars to Sclerotia stem rots in field, greenhouse and laboratory evaluations. Crop Sci., 40: 665-669.

Király, L., B. Barna. and Z. Király. 2007. Plant resistance to pathogen infection: Forms and mechanisms of innate and acquired resistance. J. Phytopathol., 155: 385-396.

Király, L., A. Künstler, R. Bacsó, Y. M. Hafez and Z. Király. 2013. Similarities and Differences in Plant and Animal Immune Systems –What is Inhibiting Pathogens? Acta Phytopathol. Entomol. Hung., 45 (1): 13-29.

Klapheck, S., I. Zimmer and H. Cosse. 1990. Scavenging of hydrogen peroxide in the endosperm of *Ricinus communis* by ascorbate peroxidase. Plant Cell Physiol., 31: 1005-1013.

Levine, A., R. Tenhaken, R. Dixon and C. Lamb. 1994. H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell 79:583-593.

Lipka, U., R. Fuchs, C. Kuhns, E. Petutschnig and V. Lipka. 2010. Live and let die-Arabidopsis nonhost resistance to powdery mildews. Eur. J. Cell Biol., 89:194-199.

O'Mahony, M. 1986. Sensory Evaluation of Food: Statistical

Methods and Procedures. CRC Press, p. 487.

Schulze-Lefert, P. and R. Panstruga. 2003. Establishment of biotrophy by parasitic fungi and reprogramming of host cells for disease resistance. Annu. Rev. Phytopathol., 41: 641-67.

Schulze-Lefert, P. and S. Bieri. 2005. Recognition at a distance. Science , 308:506-508.

Thordal-Christensen, H. 2003. Fresh insights into processes of nonhost resistance. Curr. Opin. Plant Biol., 6: 351-357.

Trujillo, M., M. Tröger, R. E. Niks, K-H. Kogel and R. Hückelhoven. 2004. Mechanistic and genetic overlap of barley host and non-host resistance to *Blumeria graminis*. Mol. Plant Pathol., 5: 389-96.

Watt, B. K. and A. L. Merrill. 1963. Composition of foods. U.S. Department of Agriculture, Agricultural Research Service, USDA .Hand Book. 8: 190.

Wu, G. S., B. J. Shortt, E. B. Lawrence, J. León, K. C. Fitzsimmons, E. B. Levine, I. Raskin and D. M. Shah. 1997. Activation of host defense mechanisms by elevated production of H_2O_2 in transgenic plants. Plant Physiol. 115:427-435.

Yulin, C., Z. Hongchang, Y. Juanni, W. Xiaojie, X. Jinrong, H. Qingmei, W. Guorong, H. Lili and K. Zhensheng. 2012. Characterization of non-host resistance in broad bean to the wheat stripe rust pathogen. BMC Plant Biology, 12:96 doi:10.1186/1471-2229-12-96.