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

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## ABSTRACT

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″. It is very important to answer the question, why non-host plants are resistant?  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 exhibited 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 (Lipka et al., 2010; Yulin et al., 2012).  NHR phenomenon explains why most of plants are immune to the enormous majority of potential 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 (Dangl and Jones 2001; Schulze-Lefert and Bieri 2005).

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 (Thordal-Christensen, 2003, Király et al., 2007). This NHR resistance is a durable 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 conducted 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 hydrogen peroxide ($H_2O_2$) (Carver et al., 1992; Hückelhoven et al., 2001; Trujillo et al., 2004).

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 (Chen *et al.*, 2003; Abdelaal *et al.,* 2014). 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 massive and economic damages of these host/pathogen interactions, research into the functional and characterization of new resistance mechanisms are needed. 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 (Hafez et al., 2007, Hafez et al., 2011). 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

### 2. 1. 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 sativum* 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.

## 2. 2. 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 (Hafez, 2009).

*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).

## 2.3. 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:

$$\text{DSI} = \frac{\sum \text{Ratings of each plant}}{10 \times \text{Number of plants rated}} \times 100$$

Histochemical staining for $O_2^-$ production in leaf tissue was based on the ability of $O_2^-$ to reduce nitro blue tetrazolium (NBT). $O_2^-$ 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 $O_2^-$ 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.

## 2. 5. 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 leaf material 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.

## 2. 6. 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 (Fig. 1 and Table 1).

Disease symptoms were also significantly appeared and visible in the host/pathogen combinations compared to the non-host which no symptoms appeared (Fig. 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).
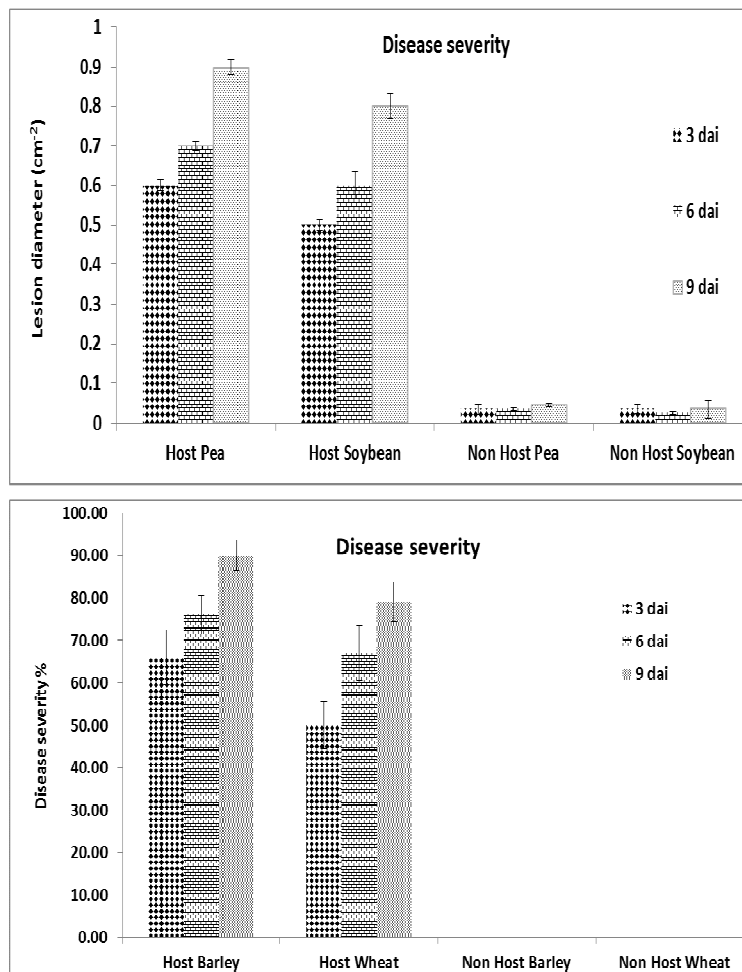
**Fig. (1): Disease severity percentage (%) of host and non-host/pathogen combinations 3, 6 and 9 days after inoculation (dai) of pea, soybean, barley and wheat plants. Host Pea: leaves inoculated with *Botrytis cinerea*. Host soybean: leaves inoculated with *Alternaria solani*. Non Host Pea: leaves 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***

**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**

**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 (Fig. 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 (Figs. 3, 4 and 5).  It is known that there is a definite correlation between ROS accumulation and inhibition of plant pathogens in resistant (immune) plants, the cause-and-effect relationship between these two events (i.e. ROS accumulation and disease resistance) is not entirely clear so far (Kiraly et al., 2103). In these results, the author has observed that in a series of plant/pathogen interactions, the "non-host" type of resistance is associated with an early activation of $O_2^{\cdot-}$ and $H_2O_2$. This accumulation in resistant non-host plants, could inhibit or kill pathogens early after inoculation (Figs. 3, 4 and 5). These results indicated that the "non-host" type of resistance recognized in pea inoculated with *PRSV* virus, soybean inoculated with *B. cinerea*, barely inoculated with wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*) and wheat inoculated with barley powdery mildew (*Blumeria graminis* f. sp. *horei*). As is seen pea, soybean, barley and wheat are incompatible (non-hosts) for these pathogenic fungi which resulted in resistance with inhibition of the pathogen and suppressed symptoms, but are compatible (hosts) with another pathogens which resulted in susceptibility with development of typical disease symptoms and unarrested growth of the pathogen (Table, 1).  Accordingly, the cause of symptomless non-host resistance could be attributed to the early inhibition or killing of infectious agents by ROS. However, in host plants exhibiting susceptibility, pathogen growth is uninhibited and consequently disease symptoms are produced possibly because there is no ROS accumulation after infection which could be due to elevated antioxidant capacity of the host early after inoculation (El-Zahaby et al., 1995; Harrach et al., 2008).

**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 (Figs. 6 and 7).
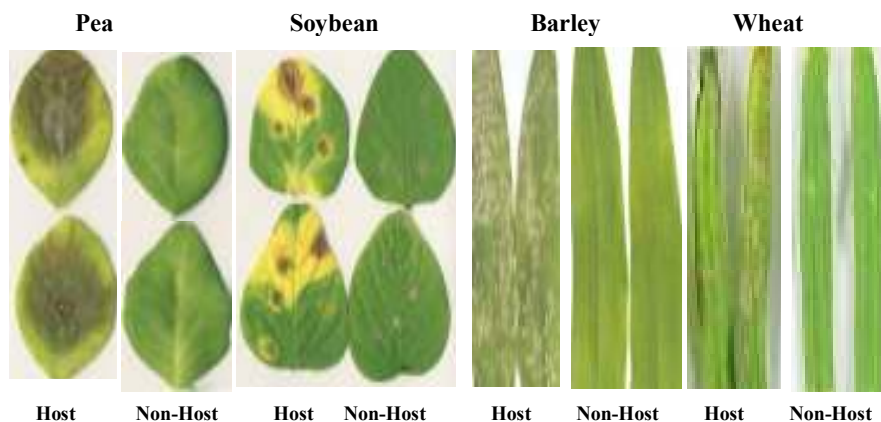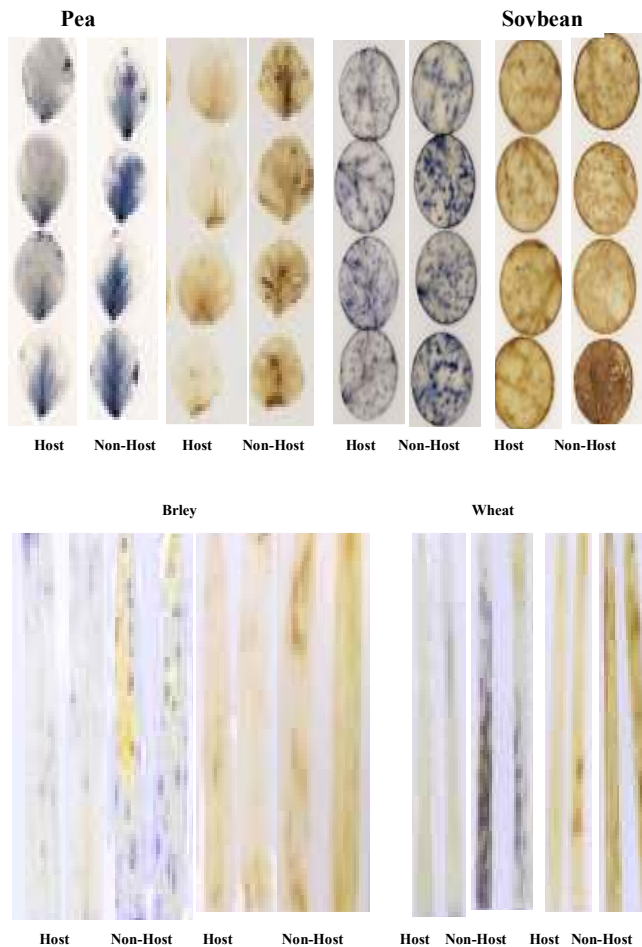
| Pea | Soybean | Barley | Wheat |
|---|---|---|---|



| Host | Non-Host | Host | Non-Host | Host | Non-Host | Host | Non-Host |
|---|---|---|---|---|---|---|---|

**Fig. (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***
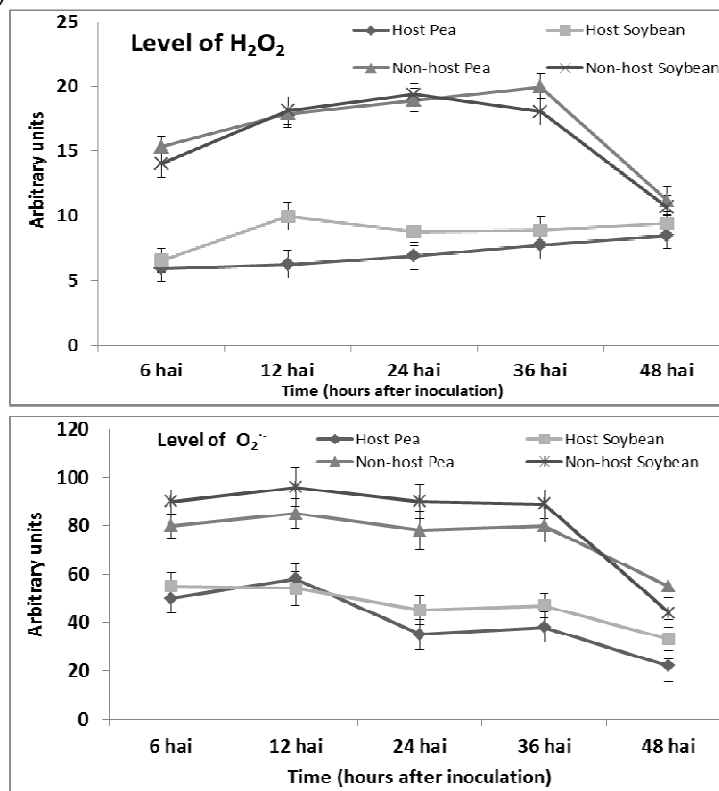
**Pea**                                    **Sovbean**



Host    Non-Host    Host    Non-Host          Host    Non-Host    Host    Non-Host

**Brley**                        **Wheat**



Host    Non-Host    Host    Non-Host          Host    Non-Host    Host    Non-Host

**Fig. (3): Purple discoloration of superoxide ($O_2^{.-}$) 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***

One can say that in these non-host resistant pea, soybean, barley and wheat plants the early accumulation of $O_2^{.-}$ 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.
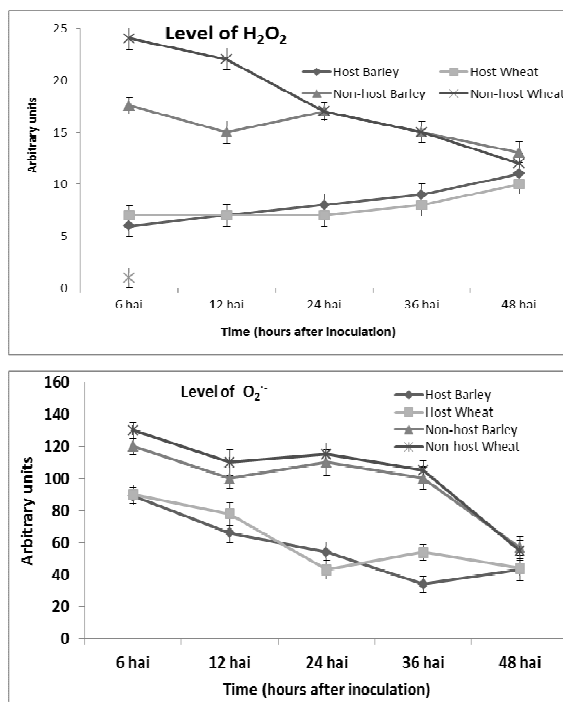
Our results are supported by our previous results in which pointed out that under natural conditions the up-regulation of antioxidant defense systems seems to be a general response to oxidative stress (Hafez et al., 2012).



Fig. (4): Levels of hydrogen peroxide ($H_2O_2$) and superoxide ($O_2^{.-}$) 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*

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).



**Fig. (5): Levels of hydrogen peroxide ($H_2O_2$) and superoxide ($O_2^-$) 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***
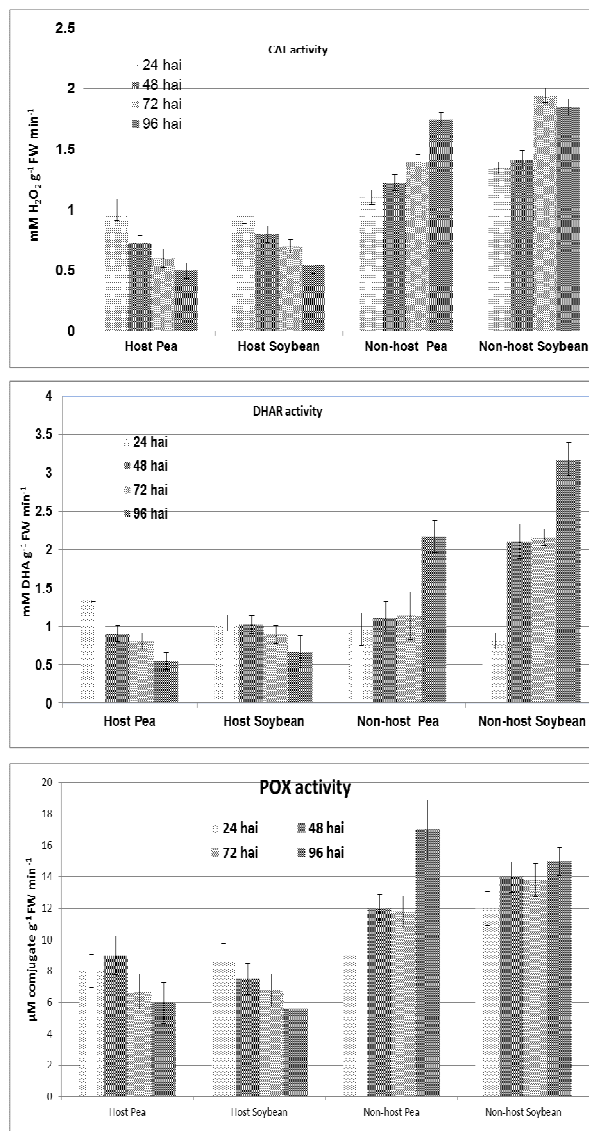
**Fig. (6): Activities of antioxidant enzymes catalase (CAT), dehydroascorbate reductase (DHAR) and peroxidase (POX) in host and non-host/pathogen combinations 24 48, 72 and 96 hours after inoculation (hai) in pea and soybean plants. Host Pea: leaves inoculated with *Botrytis cinerea*. Host soybean: 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***
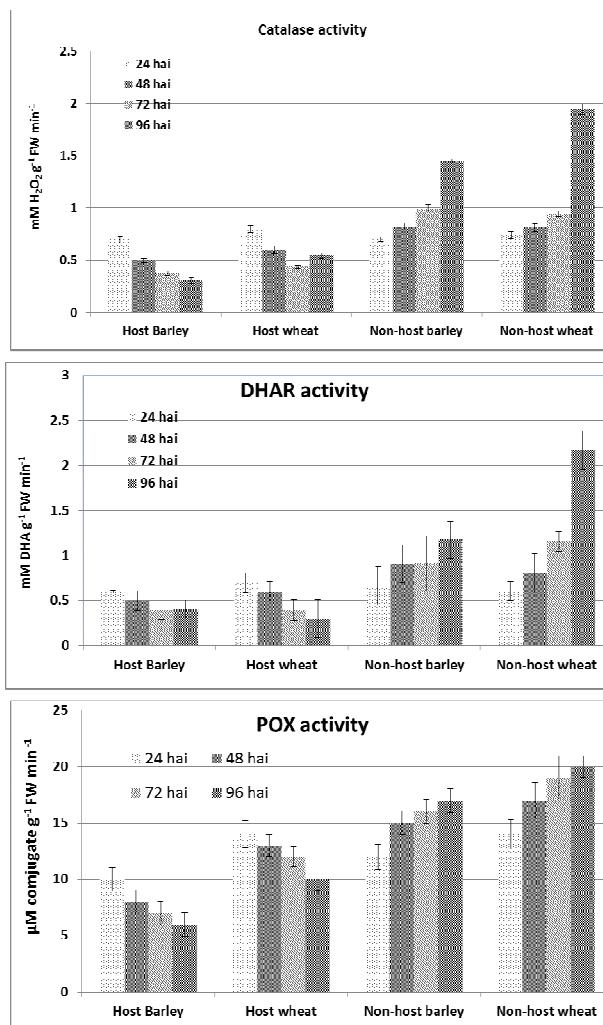
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**Fig. (7): Activities of antioxidant enzymes catalase (CAT), dehydroascorbate reductase (DHAR) and peroxidase (POX) in host and non-host/pathogen combinations 24 48, 72 and 96 hours after inoculation (hai) in barley and wheat plants. Host Pea: leaves 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***

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 (Levine et al., 1994; Wu et al., 1997; Hafez et al., 2012).

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. The author was able to characterize new histochemical and biochemical mechanisms which explain partially the question: why non-host plant is resistant to the incompatible pathogen? One can answer this puzzling question by saying that, as a result of inoculation of non-host plant with incompatible pathogens, ROS mainly superoxide and hydrogen peroxide accumulated early 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.

In summary, the author recommends the researchers and plant breeders to give more attention to these interesting new results to find new strategies for future integrated pest management practices and sustainable crop protection.

**Aknowledgement**

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## الدور الحيوي للتراكم المبكر لمشتقات الأكسجين الحرة في ميكانيكيات المقاومة غير المضيفة في النباتات البقولية والحبوب عند العدوى بمسببات الأمراض غير المتوافقة

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معظم النباتات في ظل الظروف الطبيعية تكون مقاومة لمعظم مسببات الأمراض غير المتوافقة (إصابات فيروسية، فطرية وبكتيرية). وهذا الظاهرة تسمى "المقاومة غير المضيفة (NHR) ". ومن المهم جدا الرد على هذا السؤال، لماذا النباتات غير المضيف مقاومة؟

نتيجة لاصابة النباتات البقولية (البازلاء وفول الصويا) والحبوب (الشعير والقمح) بمسببات الأمراض المتوافقة وغير المتوافقة، لوحظت أعراض مقاومة قوية جدا في حالة النباتات غير المضيفة (مقاومة) بالمقارنة مع النباتات المضيفة (المتوافقة) التي أظهرت القابلية للاصابة (حساسة).

وقد لوحظ أن مستويات مشتقات الأكسجين الحرة (ROS) وخاصة فوق اكسيد الهيدروجين ($H_2O_2$) والسوبراكسايد ($O_2^{\cdot-}$) زادت وارتفعت بشكل كبير في وقت مبكر ٦ و ١٢ و ٢٤ و ٣٦ ساعة بعد الاصابة (العدوى) في النباتات غير المضيفة بالمقارنة مع النباتات المضيفة. ومن المثير للاهتمام أن أنشطة الإنزيمات المضادة للأكسدة مثل الكتاليز (CAT)، الديهيدرواسكورببت ريدوكتيز والبيروكسيديز (POX) لم تكن زيادة كبيرة في نفس الوقت المبكر ٦-٣٦ ساعة بعد العدوى في النباتات غير المضيفة، ومع ذلك زادت هذه الإنزيمات زيادة كبيرة في وقت لاحق عند ٤٨ و ٧٢ و ٩٦ ساعة بعد العدوى في النباتات غير المضيفة بالمقارنة مع النباتات المضيفة. ويبدو أن تراكم فوق اكسيد الهيدروجين ($H_2O_2$) والسوبراكسايد ($O_2^{\cdot-}$) في وقت مبكر يمكن أن يكون له أدوار مزدوجة، الدور الاول هو تثبيط أو قتل مسببات الأمراض في وقت مبكر في النباتات غير المضيفة، والدور الثاني هو زيادة مناعة النباتات غير المضيفة من خلال تحفيز أنشطة الإنزيمات المضادة للأكسدة في وقت لاحق وبالتالي كبح إستمرار التأثير الضار من ال ROS، وبالتالي قمع أعراض المرض. ويجب إعطاء المزيد من الاهتمام لهذه الآليات والميكانيكيات الجديدة للمقاومة غير المضيفة وخاصة فيما يتعلق بمستويات ال ROS وأنشطة الانزيمات المضادة للأكسدة حيث أنها مهمة جدا لمربي النباتات ومفيدة للوصول لإستراتيجيات المكافحة البديلة أيضا.