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Original article

Biochemical and molecular characterization of non-host resistance keys in food crops

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ABSTRACT

Generally, under normal conditions plants are resistant to many of the incompatible pathogens (viral, fungal and bacterial), and this is named "non-host resistance phenomenon". To understand this phenomenon, different types of food crops (faba bean, squash, barley and wheat) were inoculated 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). Reactive oxygen species (ROS) mostly hydrogen peroxide and superoxide were significantly increased early 24 and 48 h after inoculation (hai) in the non-host plants comparing to the host. Antioxidant enzymes activity (catalase, polyphenol oxidase and peroxidase) were not increased at the same early time 24, 48 hai in the non-host resistant and host resistant plants, however, it increased later at 72 and 168 hai. Electrolyte leakage decreased significantly in non-host resistant and host resistant/pathogen combinations. Catalase and peroxidase genes were significantly expressed in non-host resistant and in host resistant plants as compared to the host susceptible one, which did not show expression using RT-PCR technique. Furthermore, *Yr5*, *Yr18* and *Yr26* resistant genes were identified positively using PCR in all treatments either host susceptible or non-host resistant plants in which prove that no clear role of these resistant genes in resistance. Early accumulation of ROS could have a dual roles, first role is preventing the growth or killing the pathogens early in the non-host, second, stimulating the gene appearance of related genes in addition the activition of antioxidant enzymes later on which thereby, neutralize the harmful effect of ROS and consequently suppressing disease symptoms. The new finding from this study supporting the plant breeders with new source of resistance to develop new resistant cultivars and/or stop the breakdown of resistance in resistant cultivars.

1. Introduction

Non-Host resistance (NHR) is defined as resistance from plants to many incompatible microbial-pathogens (Mysore and Choong-Min, 2004; Thordal-Christensen, 2003). The NHR is demonstrated in several obstacles, such as cell wall thickness, surface waxes of cuticle, plant surface topology, actin microfilaments, glucosinolate metabolism production, lignin formation, phytoalexins production, and pathogenesis-related proteins stimulation (Lee et al., 2017; Künstler et al., 2018). As a result of initial response of plant defense, the NHR against the pathogens showed two different types of reactions. The first type does not appear any visible symptoms and called plant-triggered immunity (Boller and Felix, 2009; Niks and Marcel, 2009). The second type of NHR observes several hypersensitive responses (HR) with necrosis (Gill et al., 2015; Lee et al., 2017).

Many studies have reported the important keys of NHR in plants and one of these is ROS (Cheng et al., 2012). The first study

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on the role of ROS mainly hydrogen peroxide (H_2O_2) was throughout NHR second type showed the accumulation of H_2O_2 against plant pathogenic bacteria (*Pseudomonas* spp.), which appeared strong form of resistance (Yoda et al., 2009). Further study emphasized the key role of H_2O_2 generation of plant organs such as peroxisomes and chloroplasts in HR-associated with NHR to the pathogens (Rojas et al., 2012). The H_2O_2 and superoxide (O_2) are examples for ROS can be very injurious to organisms and tissues due to their oxidized role in living resulting in the peroxidation of plasma membranes (Abdelaal et al., 2018; Hafez and Kh, 2015), and decreasing the enzymes inactivity (Fucci et al., 1983). The O_2 and H_2O_2 accumulated early in non-host pepper/*Xanthomonas campestris* pv. *vesicatoria* interaction as compared to the host/pathogen interaction in which O_2 and H_2O_2 were not accumulated (Kwak et al., 2009). Moreover, overexpression of genes such as NADPH oxidase gene which limit superoxide generation, leads to inhibit the HR-associated with NHR against bacterial and fungal pathogens (An et al., 2017). The ROS plays two main important roles throughout defense to infections. The first one is accumulation of ROS that gave inhibition/killing of pathogens with endorsing hyper sensitive necrosis (Hafez et al., 2012). The second role is decreasing of ROS levels, which encourage the antioxidant activation and resistant genes expression in the tissues next to the infection sites (Hafez et al., 2012; Künstler et al., 2018). ROS mostly, H_2O_2 plays a double role by motivating localized cell death of the host and the pathogen and stimulating the antioxidant activities as well as induces the overexpression of resistance genes (Hafez et al., 2012; Wu et al., 1997). Therefore, the current study designed to investigate the NHR mechanisms correlated with ROS levels, activity of antioxidant enzyme and the gene expression of resistant genes, consequently providing new sources of resistance to plant breeders to develop new resistant cultivars.

2. Materials and methods

2.1. Materials

Faba bean cv. (Giza-40 and Sakha-2); Wheat cv. (Morocco and Misr-2); Barley cv. (Giza-123 and Giza-131); and Squash cv. (Eskandarani) were used in this study. The seeds and grains of these plant types were obtained from Agricultural Research Center (ARC) Sakha, Kafrelsheikh, Egypt. The germination of the seeds and artificial inoculation of the pathogens were done according to the method described by (Chris, 2012; Hafez et al., 2003). Research was conducted at green house and labs. of Botany Department, Faculty of Agriculture, Kafr-Elsheikh University, Egypt, as well as Center of Excellence (CEBR), King Saud University, Saudi Arabia.

2.2. Plant pathogens

Papaya ring spot virus (PRSV) Egyptian isolate was kept on squash (host). For virus inoculation, infected leaves were regulated in water. Carborundum was applied as abrasive for virus as well as mock inoculations. Both *Bgh* and *Bgt* caused wheat powdery mildew were used. Inocula of powdery mildews were dispersed by placing sporulating colonies of both pathogens beneath ventilation fans (Hafez et al., 2003). Barley net blotch caused by *Pyrenophora teres* (*Pt*), (*Bf*) and faba rust caused by *Uromyces fabae* (*Uf*) spores were obtained from FCRI Research Institute, Egypt. Spores were harvested from susceptible Morocco cultivar. Mixture of fresh spores and talcum 1:5 (w/w), then, second leaves of 16 days seedlings were inoculated. 5 replicate pots of seedlings were inoculated for each cultivar under greenhouse in a spore-proof cabin (Chris, 2012).

2.3. Detection of $O_2^{\cdot-}$ and H_2O_2

The $O_2^{\cdot-}$ and H_2O_2 were envisaged with purple and brown colour, respectively. It was measured at 24, 48, 72 and 168 (hai) of faba bean, squash, wheat, and barley plants. While barley, faba bean, squash and wheat leaves were penetrated with potassium phosphate buffer containing 0.1% NBT or 0.1% DAB staining (Adam et al., 1989). Samples incubated between 20 and 120 mins, after that, clean in 0.15% of TCA (trichloroacetic) mixed with 4:1 of ethanol: chloroform for 24 h (Hückelhoven et al., 1999). Samples were positioned in 50% glycerol. Discolorations were tested by a ChemiImager program. The test was repeated 3 times.

2.4. Biochemical assays of antioxidant enzymes

Half gram fresh leaf was grinding at 4 °C in 3 ml of 50 mM TRIS buffer in which contain 1 mM EDTA-Na_2 as well as 7.5% pvp at 24,48,72 and 168 (hai) of faba bean, squash, wheat and barley plants. Centrifugation of samples at 12,000 rpm for 20 min under 4 °C, total soluble enzyme activities were determined in the supernatant three times (Hafez, 2010). The catalase (CAT), polyphenoloxidase (PPO) and guiacol peroxidase (POX) enzyme activities were measured (Aebi, 1984; Hammerschmidt and Nuckles et al., 1982). Every 30 sec, changes in absorbance were recorded with 3 min intervals.

2.5. Electrolyte leakage percentage (EL%)

The EL % were determined at 24,48,72 and 168 (hai) of faba bean, squash, wheat and barley plants with some modification. 50 g of leaves were placed individual into 50 ml deionized water.

Table 1
Sequences of primers that used in this investigation.

Genes	Marker Name	Primer sequence	Ta (°C)	Amplicon size (bp)	Reference
Yr 5	STS7 STS8	F5′-GTACAATTCACCTAGAGT-3′ R5′-GCAAGTTTTCTCCCTAT-3′	45	478	Chen et al. (2003)
Yr 18	L34DINT9-F L34PLUS-R	F5′-TTGATGAAACCAGTTTTTTTTCTA-3′ R5′-GCCATTTAACATAATCATGATGGA-3′	58	517	Lagudah et al. (2009)
Yr 26	WE 173	F5′-GGGACAAGGGGAGTTGAAGC-3′ R5′-GAGAGTTCCAAGCAGAACAC-3′	55	451-730	Wang et al. (2008)
Actin		F5′-GAAGCTGCAGGTATCCATGAGACC-3′ R5′-AGGCAGTGATCTCCTTGCTCATC -3′	55.3	151	Padaria et al., 2014
Peroxidase (PR9)		F5′-ATCAGACCGTCTCCTGCG-3′ R5′-GCAGCTGAGCCTGATCTG-3′	51.5	750	Cawood et al., 2013
Catalase (CAT)		F5′-ACTACGACGGGCTCATG-3′ R5′-GGAGCTGACACGGCTTC-3′	48.6	870	Luna et al., 2004

Samples were shakening for 20 h to leakage the electrolyte from tissues. Measurements were recorded using Acromet AR20 electrical meter for conductivity. Afrer that flasks immersed for 1 hr in water bath (80 °C) to induce cell burst, then, vials shaked for 20 h under 21 °C. Conductivity determined for flasks individually. EL% for each sample recorded: initial conductivity /final conductivity × 100.

2.6. Genomic extraction and resistance genes amplification

Genomic DNA of wheat seedlings was extracted from 60 mg of fresh leaves using Invisorb® Mini Kit according to manufacturer's instructions. The genomic regions of resistance genes *Yr5, Yr18, Yr26* using gene specific primers (Table 1) were detected in thermal cycler PCR. Genes amplification was done following the PCR

Table 2
Reaction of host and non-host/pathogen combinations.

Plants	Host	Results	Non-host	Results
Faba bean squash	*Botrytis fabae*	S	Papaya rings pot virus	R
Barley	Papaya ring spot virus	S	*Puccinia striiformis* f.sp. *tritici*	R
Wheat	*Blumeria graminis* f. sp. *hordei*	S	*Blumeria graminis* f. sp. *tritici*	R
	Pyenophora teres	S	Papaya ring spot virus (PRSV)	R
	Blumeria graminis f. sp. *tritici*	S	*Blumeria graminis* f. sp. *hordei*	R
	Puccinia striiformis f.sp. *tritici*	S	*Uromyces fabae*	R

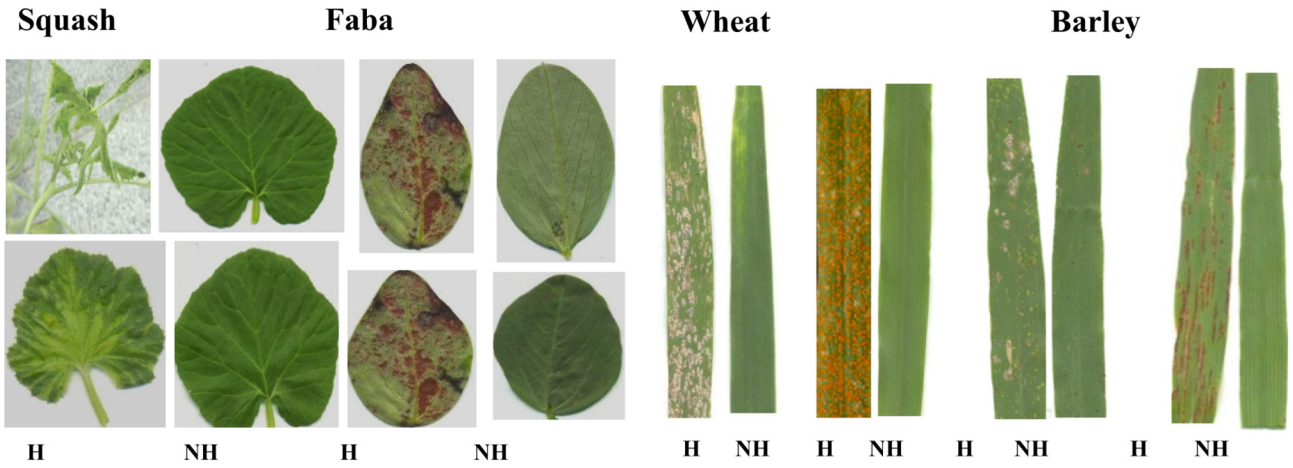

Fig. 1. Disease symptoms of host (H) and non-host (NH)/pathogen combinations 7 days after inoculation (dai) in squash, faba bean, wheat and barley plants. Host squash: inoculated leaves with *Papaya ring spot virus (PRSV)*. Host faba bean: inoculated leaves with *B. fabae* (*Bf*). Non-host Squash: inoculated leaves with *P. striiformis f.sp. tritici (Pst)*. Non-host faba bean: inoculated leaves with *PRSV*. Host wheat: inoculated leaves with *B. graminis* f. sp. *tritic (Bgt)* and *Pst*. Non-host wheat: inoculated leaves with *Blumeria graminis f. sp. hordei (Bgh)* and *Uromyces fabae* (*Uf*). Host Barley: inoculated leaves with *Bgh and Pyenophora teres* (*Pt*). Non-host Barley: inoculated leaves with *Bgt* and *(PRSV)*.

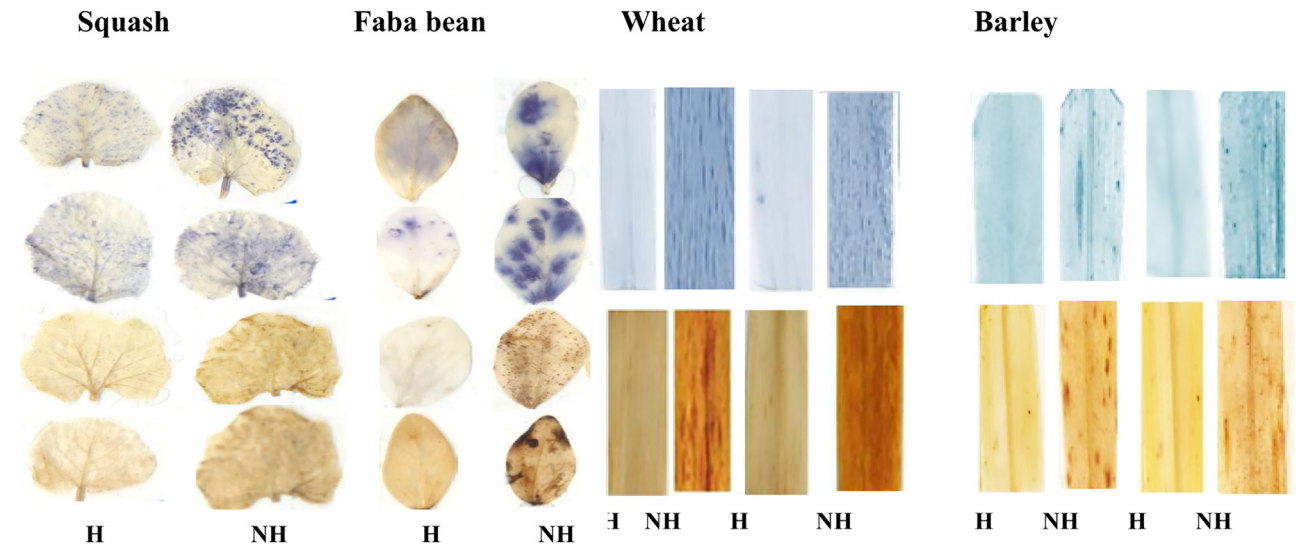

Fig. 2. Purple discoloration of $O_2^{\cdot-}$) (upper row) and brown discoloration H_2O_2 (lower row) of host (H) and non-host (NH)/pathogen combinations 24 h after inoculation (hai) in Squash, faba bean, Wheat and Barley plants. Host Squash: leaves inoculated with *Papaya ring spot virus (PRSV)*. Host faba bean: leaves inoculated with *Botrytis fabae* (*Bf*). Non-host Squash: leaves inoculated with *Puccinia striiformis f.sp. tritici (Pst)*. Non-host faba bean: leaves inoculated with *PRSV*. Host Wheat: leaves inoculated with *Blumeria graminis* f. sp. *tritic (Bgt)* and *Pst*. Non-host Wheat: leaves inoculated with *Blumeria graminis f. sp. hordei (Bgh)* and *Uromyces fabae* (*Uf*). Host Barley: leaves inoculated with *Bgh and Pyenophora teres* (*Pt*). Non-host Barley: leaves inoculated with *Bgt* and *(PRSV)*.

program with initial denaturation 94 °C for 3 min, annealing temperature was (40 °C for *Yr5*, 48 °C for *Yr18* and 50 °C for *Yr26*) for 1 min., PCR cycles were 30 cycles of 94 °C 1 min; and final extension was 72 °C for 5 min. PCR outcomes analyzed by electrophoresis (Khan et al., 2019).

2.7. RNA extraction and antioxidant genes expression

Total RNA of wheat plants was extracted form 1 g of fresh tissue using i-genomic Kits (InviTrap; Cat. No. 0515). One-Step RT-PCR Kit (InviTarp) was used. Reaction volume was 50 μl as follows: 1 μl RNA, 20 μl RNase-water, 10 μl 5 × InviTarp One-Step buffer (containing 12.5 mM $MgCl_2$), 2-μl dNTP mix (10 mM each), 2 μl InviTarp One-Step RT-PCR Enzyme Mix and 2.5 μl of each primer (Table 1). RT-PCR program was designed with initial denaturation 94 °C for 3 min, annealing temperature was (55.3 °C for Actin gene, 51.5 °C for Peroxidase gene and 48.6 °C for Catalase gene) for 1 min., PCR cycles were 40 cycles of 94 °C 1 min; and final extension was 72 °C for 5 min.

2.8. Statistical analysis

Data was presented as mean ± SD. Complete experiment was implemented in a designed output and thricely replicated

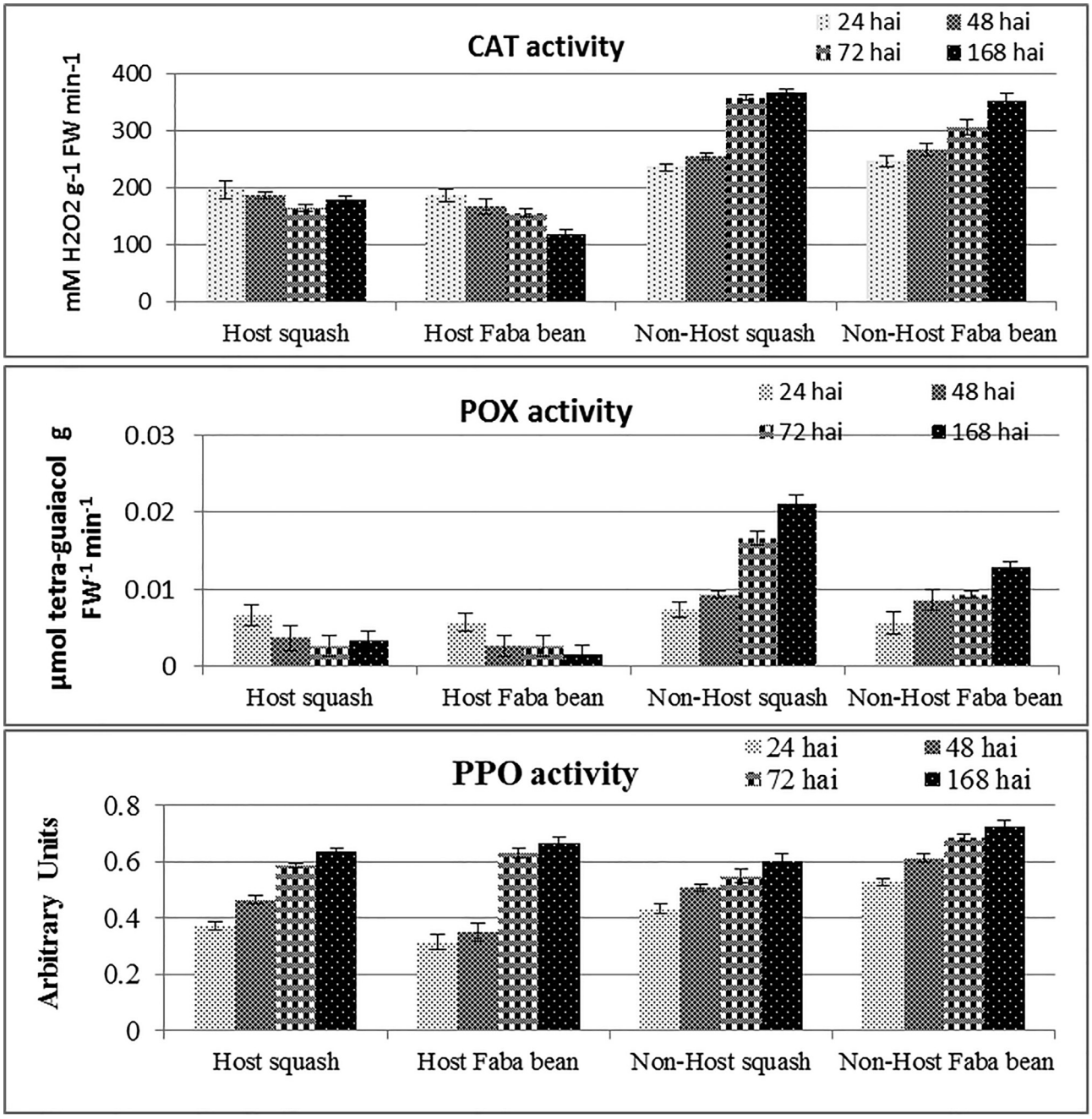

Fig. 3. Antioxidant enzymes activity (CAT, POX and PPO) in host and non-host/pathogen combinations 24, 48, 72 and 168 h after inoculation (hai) in squash and faba bean plants. Host squash**:** leaves inoculated with *Papaya ring spot virus (PRSV)*. Host faba bean**:** leaves inoculated with *Botrytis fabae* (*Bf*). Non-host squash**:** inoculated leaves with *Puccinia striiformis f.sp. tritici (Pst).* Non-host faba bean: leaves inoculated with *PRSV*.

(Mohony, 1985). The student *t*-test were regulated with the mean differences as $p < 0.05$ as statistically significant.

3. Results

3.1. Disease symptoms of host and non-host/pathogen combinations

When plants were inoculated with compatible pathogens, they become susceptible (hosts), however, when the same plants were inoculated with incompatible pathogens, they become resistant (non-hosts) (Table 2). In the "non-host" plants faba bean, squash, barley and wheat showed resistance against PRSV, *Pst, Bgt* and PRSV as well as *Bgh* and Uf pathogens, respectively in which showed resistant compared to the "host" plants faba bean, squash, barley and wheat inoculated with *Bf,* PRSV, *Bgh and Pt.* As well as *Bgt Pst* pathogens, respectively showed strong susceptibility (Fig. 1).

3.2. Combination of host/non-host pathogens

ROS mostly $O_2^{\cdot-}$ as well as H_2O_2 were accumulated significantly early 24 h after inoculation (hai) in non-host/pathogen combinations as compared to the host/pathogen combinations (Fig. 2).

3.3. Combination of antioxidant enzymes activity

The activity of CAT, PPO and POX enzymes were increased a little at 24 h after inoculation (hai) in all non-host/pathogen combi-

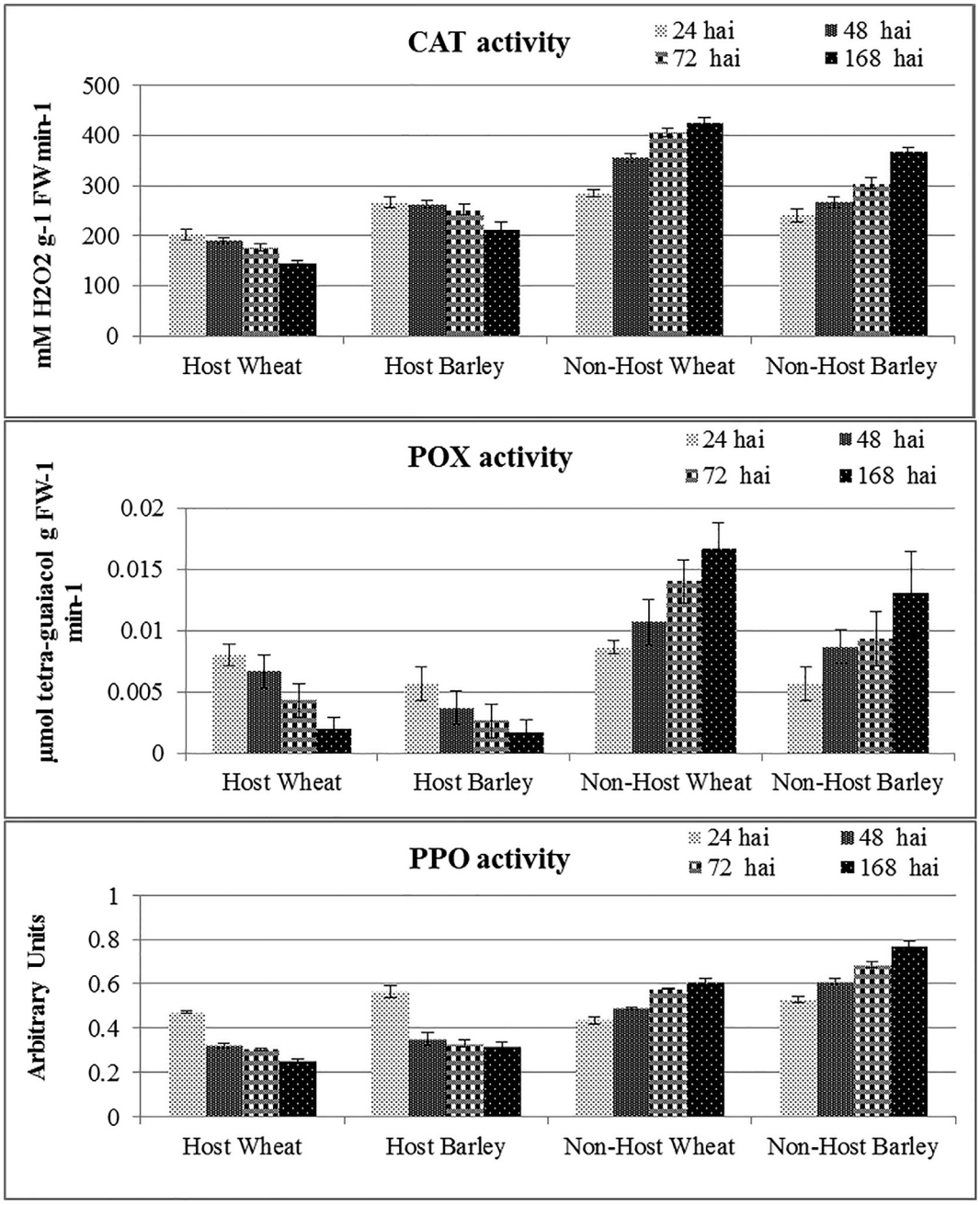

Fig. 4. Antioxidant enzymes activity (CAT, POX and PPO) in host and non-host/pathogen combinations 24, 48, 72 and 168 h after inoculation (hai) in wheat and barley plants. Host wheat**:** inoculated leaves with *Blumeria graminis* f. sp. *tritic (Bgt).* Host barley**:** inoculated leaves with *Blumeria graminis f. sp. hordei (Bgh).* Non-host wheat**:** inoculated leaves with *(Bgh).* Non-host barley**:** inoculated leaves with *(Bgt).*

nations in faba bean, squash, barley and wheat plants, however, these enzymes were increased significantly at 48, 72 and 168 hai (Figs. 3–5). In the non-host resistant faba bean, squash, barley and wheat plants, early accumulation of $O_2^{\cdot-}$ and H_2O_2 not only inhibit or kill the incompatible pathogens early hai but also motivate antioxidant enzymes activity (CAT, POX and PPO).

3.4. Combination of various pathogens electrolyte leakages

Electrolytes leakage (EL) is an indicator of the cell membrane permeability. In the "host" plants Faba bean, squash, barley and wheat inoculated with *Bf*, PRSV, *Bgh*, *Pt* and *Bgt*, *Pst* respectively, showed significant increase of the membrane permeability compared to the "non-host" plants Faba bean, squash, barley and wheat showed resistance against *PRSV*, *Pst*, *Bgt, PRSV* and *Bgh*, *Uf,* respectively, (Figs. 6–8).

3.5. Characterization of resistance genes by molecular markers.

Three amplification fragments corresponding to DNA marker of yellow rust resistance genes *(Yr 5, Yr 18 and Yr 26)* were amplified from DNA of two wheat cultivars. The markers of *Yr5*, r *Yr18* and *Yr* genes were characterized in host and non-host plants (Fig. 9). This means that, the resistant genes *Yr5*, *Yr18* and *Yr26* were identified positively using PCR in all treatments either host susceptible or non-host resistant plants.

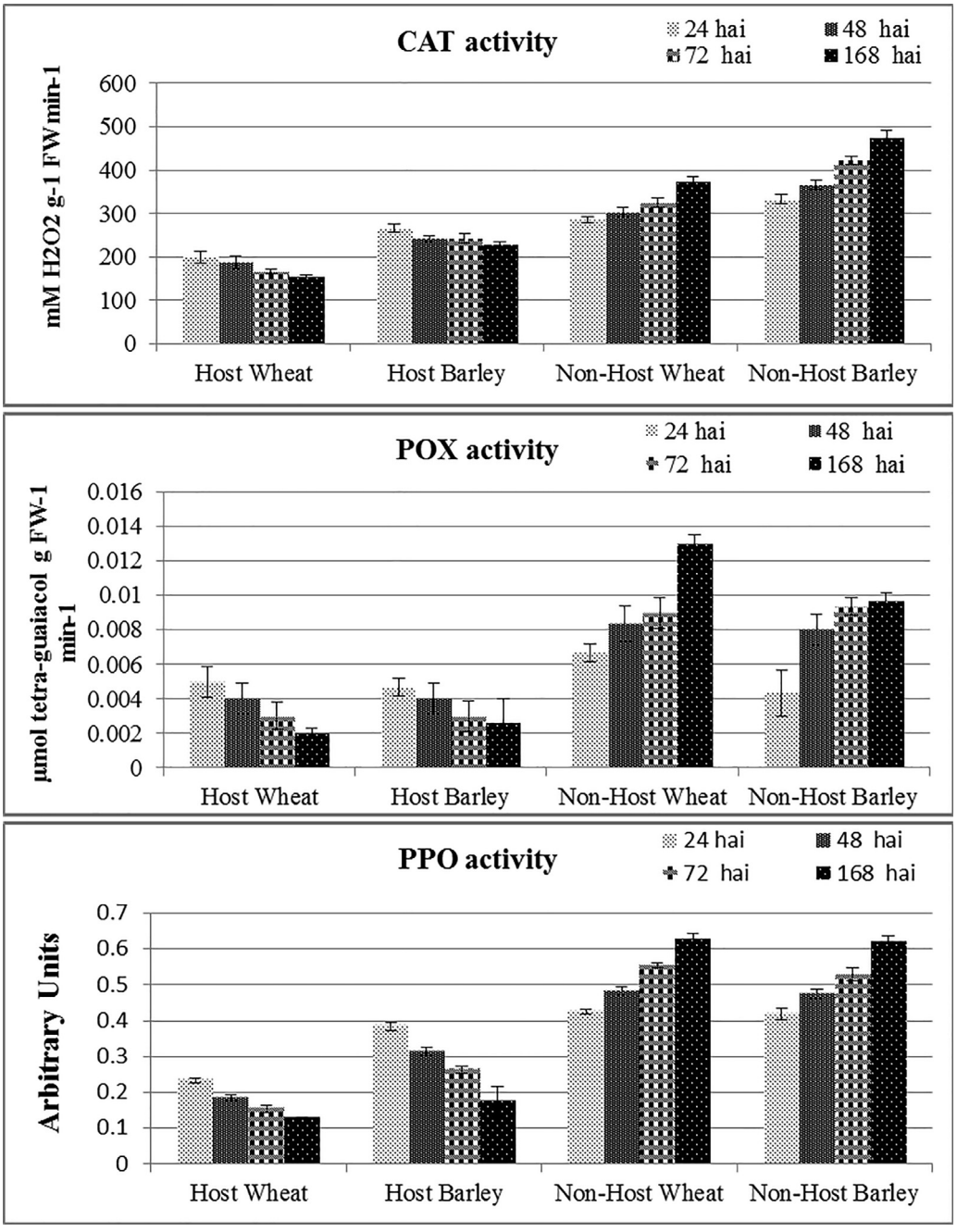

Fig. 5. Antioxidant enzymes activity (CAT, POX and PPO) in host and non-host/pathogen combinations 24, 48, 72 and 168 h (hai) after inoculation in wheat and barley plants. Host wheat**:** inoculated leaves with *Puccinia striiformis f.sp. tritici (Pst)*. Host barley**:** inoculated leaves with *Pyenophora teres* (*Pt*). Non-host wheat**:** inoculated leaves with *Uromyces fabae* (*Uf*). Non-host barley**:** inoculated leaves with *Papaya ring spot virus (PRSV)*.

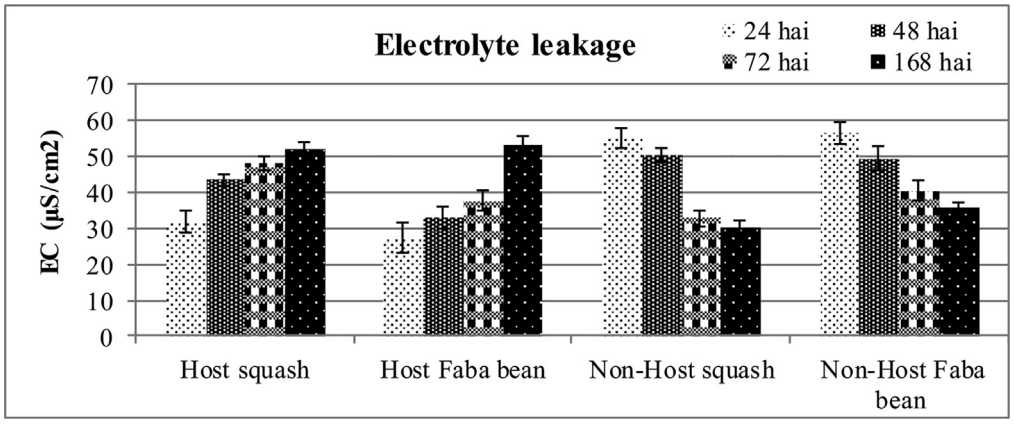

Fig. 6. Electrolyte leakage of host and non- host /pathogen combinations 24, 48, 72, 168 h after inoculation (hai) in squash and faba bean plants. Host squash**:** inoculated leaves with *Papaya ring spot virus (PRSV)*. Host faba bean**:** inoculated leaves with *Botrytis fabae* (*Bf)*. Non-host squash**:** inoculated leaves with *Puccinia striiformis f.sp. tritici (Pst).* Non-host faba bean: inoculated leaves with *PRSV*.

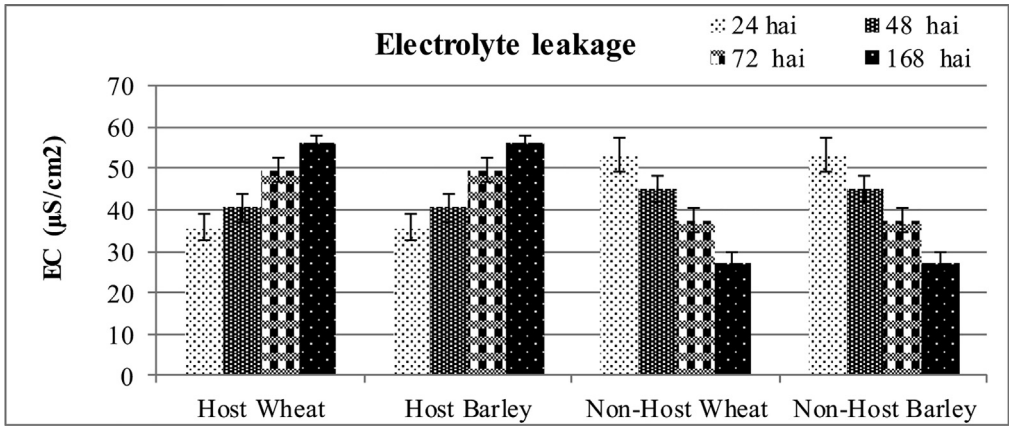

Fig. 7. Electrolyte leakage of host and non- host /pathogen combinations 24, 48, 72, 168 h (hai) after inoculation in wheat and barley plants. Host wheat**:** inoculated leaves with *Blumeria graminis* f. sp. *tritic (Bgt)*. Host barley**:** inoculated leaves with *Blumeria graminis f. sp. hordei (Bgh).* Non-host wheat**:** inoculated leaves with *(Bgh)*. Non-host barley**:** inoculated leaves with *(Bgt)*.

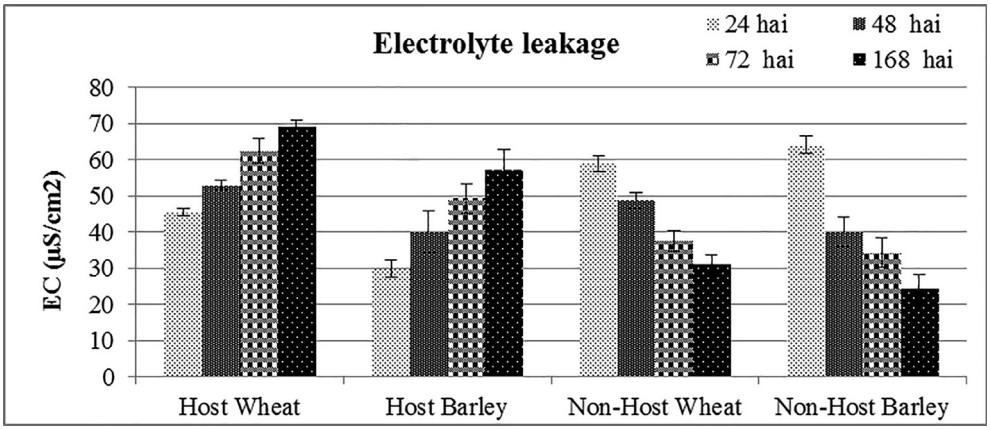

Fig. 8. Electrolyte leakage of host and non- host /pathogen combinations 24, 48, 72, 168 h after inoculation (hai) in wheat and barley plants. Host Wheat**:** leaves inoculated with *Puccinia striiformis f.sp. tritici (Pst)*. Host Barley**:** leaves inoculated with *Pyenophora teres* (Pt). Non-host Wheat**:** inoculated leaves with *Uromyces fabae*. Non-host Barley**:** inoculated leaves with *Papaya ring spot virus (PRSV)*.

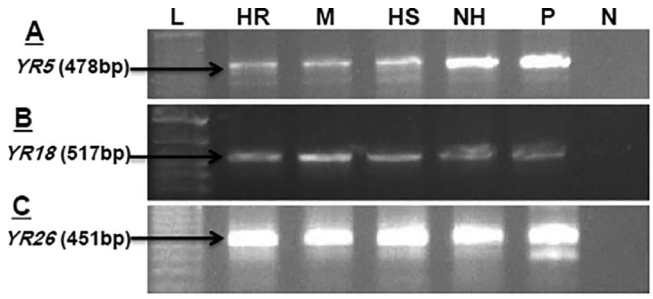

Fig. 9. Amplification results of PCR products using *Yr5* marker (478 bp); *Yr18* marker (517 bp); and *Yr26* marker (7451 bp) in host and nonhost plants. L: ladder; HR: host-resistance; M: mock; HS: host-susceptabile; NH: non-host; P: positive control of the gene; N: negative control.

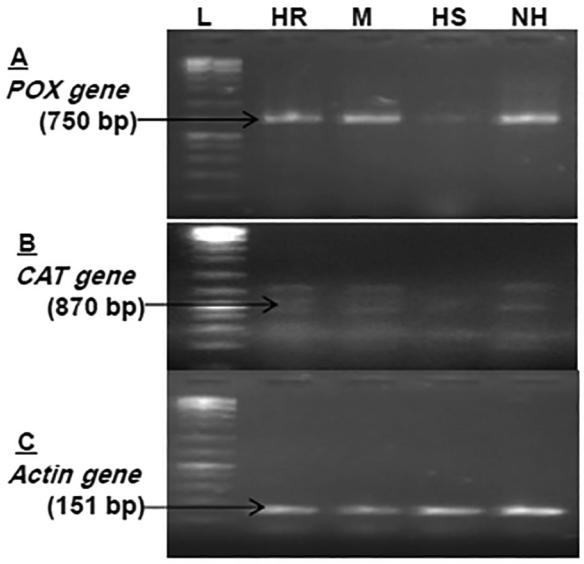

Fig. 10. RT-PCR analysis of antioxidants genes Expression. (A) Expression of peroxidase *(POX)*; (B) Expression of catalase *(CAT);* (C) Expression of *Actin* gene using as a control in host and non-host plants, 24 h after inoculation (hai).

3.6. Gene expression of enzymes in host/non-host pathogen combinations of wheat

The gene expression of peroxidase (*POX*) and catalase (*CAT*) were significantly expressed in non-host resistant and host resistant plants early after inoculation 24 h after inoculation (hai) compared with control host susceptible plants (Fig. 10). Peroxidase (*POX*) and Catalase (*CAT*) genes were significantly expressed in non-host resistant and in host resistant plants as compared to the susceptible one which did not show expression using RT-PCR technique (Fig. 10).

4. Discussion

Under the infection with the host/pathogen and non-host, the disease symptoms were significantly visible in the host/pathogen combinations compared to the non-host. In the non-host no symptoms appeared. Similarly, it has been confirmed in non-host pathogen (NHP), defines non-host plant such as turnip, which is majorly effected of *Hyaloperonospora arabidopsidis* are documented in *Arabidopsis Thaliana*, known as host for this pathogen (Fabro et al., 2011). The early production of ROS may be the cause of non-host resistant (Hafez et al., 2009). This result supports the idea that when ROS applied exogenously or any compounds producing ROS or inducing ROS at early time after inoculation, pathogens could be killed or significantly inhibited (Hafez et al., 2013). The production of ROS mainly H_2O_2 and O_2^- is an inevitable consequence of aerobic respiration. Therefore, the discoloration (brown and purple) in the treatments are indicators of H_2O_2 and O_2^- intensity or high levels, respectively, as compared with the control in which H_2O_2 and O_2^- levels were significantly decreased.

Almost 1–2% of O_2 consumption leads to the generation of O_2^- in the plant tissues, well-documented to increase H_2O_2, which leads to stress such as oxidative incidence in plant tissues (Abdelaal et al., 2014; Hafez et al., 2014). The increase of ROS in the choloroplastids occurs continuously through fractional decrease of oxygen molecules. The early accumulation of ROS in the non-host resistant plants up-regulate the antioxidants supporting the results which proved that H_2O_2 up-regulate antioxidant systems at very low levels under abiotic-stress conditions (Gechev et al., 2002). In this study, the ROS mainly O_2^- and H_2O_2 significantly accumulated early at 24 h after inoculation (hai) in all non-host/pathogen combinations in faba bean, squash, barley and wheat plants. In the non-host/pathogen combination, when wheat infected with barley powdery mildew, the O_2^- and H_2O_2 were accumulated early after inoculation and this early induction may be the cause of non-host resistant (Hafez et al., 2009). This result demonstrated that application of ROS or any compounds producing or inducing ROS at early time after inoculation, pathogens could be killed or significantly inhibited.

Enzymatic antioxidants include many enzymes such as catalase and peroxidase. In this study, the CAT, POX and PPO activities were significantly elevated in leaves of barley treated with fungicides and the non-traditional treatments, compared with control treatments. The CAT, POX and PPO activities were increased a little at 12 and 24 h after inoculation (hai) in all non-host/pathogen combinations in faba bean, squash, barley and wheat plants. Nevertheless, these enzymes were increased significantly at 48, 72 and 168 hai. This result might be due to the key role of ROS and antioxidants in the mechanisms of non-host resistance (Hafez and Kh, 2015). We can say that in these non-host resistant faba bean, squash, barley and wheat plants, the early production of H_2O_2 and O_2^- not only inhibit or kill the incompatible pathogens at 6–48 hai but also stimulate the antioxidant enzymes activities (CAT, POX and PPO) alter at 24–168 hai. These results are supported by our previous findings (Omara and Khaled, 2018). In the "host" plants showed significant increase in EL% compared to the "non-host" plants. Analogous results were documented in barley plants under drought stress (Abdelaal et al., 2018) and in wheat plants under leaf rust disease (Omara et al., 2019). This might result in the loss of host cells constituents, which may be used as a source of nutrients by the pathogen. Our results also indicated that these treatments protected the membranes of barley during the pathogen attack, while the membranes of the susceptible cultivar were affected by the pathogen infection and lost its constituents.

Three amplification fragments corresponding to DNA markers of yellow rust resistance genes *(Yr5*, *Yr*18 and *Yr*26) were amplified from DNA of host and non-host plants. The results showed that all these three genes were presented in host and non-host plants. This indicated that these resistant genes have unclear/or no role in non-host resistance mechanisms. Hydrogen peroxide can regulate the genes expression, including genes encoding antioxidants in addition to genes leading to H_2O_2 production (Desikan et al., 2001).

5. Conclusion

It can be concluded that the new histo-chemical, biochemical and molecular mechanisms of non-host resistance were found by

the authors in this study. The early accumulation of ROS mainly $O_2^{\cdot-}$ and H_2O_2 increase the antioxidant enzymes activities later and overexpression of related genes, which thereby could immunize plants by suppressing disease symptoms and counteract the injurious 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 food crops production.

Declaration of Competing Interest

None.

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