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Role of Reactive Oxygen Species (ROS) in Non-host Resistance Against Phytopathogens

Hafez, Y.M.[1]; Abdelaal, K.A.A.[1]; Elbaghdady, N.A.A.[2] and Omar, A.F.[1]

1- *Excellence Center (EPCRS), Plant Pathology and Biotechnology Lab, Agricultural Botany Department, Faculty of Agriculture, Kafrelsheikh University, 33516 Kafrelsheikh, Egypt.*

2- *Genetics Department, Faculty of Agriculture, Kafrelsheikh University, 33516 Kafrelsheikh, Egypt.*

The majority of the plants in the natural conditions are resistant to most of the incompatible pathogens (viral, fungal and bacterial infections). This phenomenon is called "non-host resistance". This type of resistance is very important however, not enough research was conducted to explain the mechanism of this type of resistance.

When the authors inoculated several non-host plants with incompatible pathogens, levels of hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\cdot -}$) were increased and elevated early after inoculation. This phenomenon was found in tomato, datura, tobacco, cucumber, squash and Chenopodium which were inoculated with tobacco powdery mildew, *Papaya ringspot virus* (PRSV), cucumber powdery mildew, tobacco mosaic virus (TMV), tomato powdery mildew and PRSV, respectively.

Interestingly that, when some hosts from those mentioned above were inoculated, each with its compatible pathogen, no accumulation of H_2O_2 and $O_2^{\cdot -}$ was occurred.

The authors concluded that reactive oxygen species (ROS) mainly H_2O_2 and $O_2^{\cdot -}$ could have a key role in inhibiting or killing the pathogens early in the non-host plants. The authors recommend giving more attention to the application of H_2O_2 and $O_2^{\cdot -}$ either with direct application or with applying compounds which induce or produce ROS against phytopathogens.

Keywords: Cucumber, phytopargogens, non-host resistance, reactive oxygen, squash, tobacco and tomato.

The non-host resistance of plants to pathogens mainly viral, bacterial and fungal infections can be defined as an innate non-specific resistance which effective against all known isolates of several species of the pathogens (Thordal-Christensen, 2003, Király *et al.*, 2007). This type of 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 and Trujillo *et al.*, 2004).

Until now, almost no experimental results have been achieved which would explain the question: what is arresting or killing the pathogen 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).

The aim of this research was to clarify the mode of action or the non-host resistance mechanisms and its relations with reactive oxygen species (ROS) such as superoxide and hydrogen peroxide.

Materials and Methods

Plant hosts:

Tomato (*Lycpersicon esculentum*), datura (*Datura stramonium*), tobacco (*Nicotiana tabacum*), cucumber (*Cucumis sativus*) and squash (*Cucurbita pepo*), seeds were sown into soil and grown under greenhouse conditions. Temperature was 18-23°C, with a 16-hour photoperiod per day using supplemental light with a light intensity of 160 µE m^{-2} s^{-1} and a relative humidity of 75-80%.

Plant pathogens:

Tobacco powdery mildew *Golovinomyces orontii* strain BP-1TOB Hungarian isolate, *Papaya ringspot virus* (PRSV) Egyptian isolate, cucumber powdery mildew *Podosphaera xanthii* Hungarian isolate, *Tobacco mosaic virus* (TMV) Hungarian isolate and tomato powdery mildew *Oidium neolycopersici* strain BP-P5 (provided by L. Kiss, cf. Kiss *et al.*, 2001), were maintained on the other hand under greenhouse conditions and were used for all inoculation experiments.

Fungal and viral inoculations:

Powdery mildew inocula were dispersed in the greenhouse atmosphere by placing plants of barley bearing sporulating colonies of *Bgh* beneath ventilation fans of the greenhouse (Hafez and Kiraly, 2003). The TMV and PRSV were maintained on the host susceptible cultivars of tobacco (*Nicotiana tabacum*) and squash (*Cucurbita pepo*), respectively. For mechanical virus inoculation, viral-infected leaves were homogenized in tap water. Carborundum was used as an abrasive for both virus and mock inoculations (Hafez, 2009).

Histochemical analysis of superoxide ($O_2^{\,-}$):

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) 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, leaves were stored in 50% glycerol for evaluation.

Histochemical analysis of hydrogen peroxide (H_2O_2):

Leaves were infiltrated with 0.1% 3, 3-diaminobenzidine (DAB) in 10 mM tris buffer (pH 7.8) for histochemical detection of H_2O_2. 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 16, 20, 24, 48, and 72 hours after infection.

Results and Discussion

Rresults indicate that when some plants were inoculated with some compatible pathogens, they became susceptible hosts however, when the same plants were treated with incompatible pathogens, they became non-hosts resistant to these incompatible pathogens (Table, 1).

Tomato/Tobacco powdery mildew combination :

Accumulation of $O_2^{\cdot-}$ in the non-host tomato leaves inoculated with tobacco powdery mildew (*Golovinomyces orontii*) occurred 24 hours after inoculation, as compared to tomato inoculated with the tomato powdery mildew (*Oidium neolycopersici*), where $O_2^{\cdot-}$ did not accumulate (Table, 1 and Fig. 1).

Datura/ Papaya ringspot virus (PRSV) combination:

As a result of inoculation of non-host datura with an inappropriate *Papaya ringspot virus* (PRSV), the production and accumulation of $O_2^{\cdot-}$ occurred sometimes early, at 24 h after infection in some preliminary experiments as compared to the host datura inoculated with TMV (Fig., 1).

Tobacco/Cucumber powdery mildew combination :

Significant accumulation of $O_2^{\cdot-}$ in the non-host tobacco leaves inoculated with cucumber powdery mildew (*Podosphaera xanthii*) occurred 24 hours after inoculation. However, in the same tobacco cultivar inoculated with tobacco powdery mildew (*Golovinomyces orontii*), $O_2^{\cdot-}$ did not accumulate at all (Table, 1 and Fig., 1).

Level of superoxide ($O_2^{\cdot-}$) in the non-host/pathogen combinations
Cucumber/Tobacco mosaic virus (TMV) combination

Accumulation of $O_2^{\cdot-}$ in the non-host cucumber leaves inoculated with TMV occurred 24 hours after inoculation. On the other hand, in cucumber inoculated with the appropriate powdery mildew (*Podosphaera xanthii*), $O_2^{\cdot-}$ did not accumulate (Table 1 and Fig. 1).

Squash/Tomato powdery mildew combination :

Accumulation of $O_2^{\cdot-}$ in the non-host squash inoculated with tomato powdery mildew (*Oidium neolycopersici*) occurred slightly at 24 hours after inoculation in some preliminary experiments (Fig., 1).

Chenopodium/ Papaya ringspot virus (PRSV) combination:

Accumulation of O2.$^{-}$ in the non-host Chenopodium inoculated with PRSV occurred 24 hours after inoculation in some preliminary experiments (Fig., 1).

Level of hydrogen peroxide (H_2O_2) in the non-host/pathogen combinations:

Accumulation of H_2O_2 in tomato inoculated with tobacco powdery mildew, squash inoculated with tomato powdery mildew and cucumber inoculated with Tobacco mosaic virus (TMV) combinations occurred at 36 hours after inoculation. In the other non-host pathogen combinations, accumulation was not significant (Fig., 1).

One can suggest that reactive oxygen species (ROS) may have a pivotal role in the arrest of pathogens in non-host plants. It was found that the levels of superoxide and hydrogen peroxide were higher in the non-host resistant plants inoculated with inappropriate pathogens than in compatible host/pathogen combinations. These results supported the ideas that superoxide is arresting the invading of pathogens in non-host plants (Hafez *et al*., 2007) and enhanced in the non-host resistant plants (Künstler *et al*., 2008). This elevated level of hydrogen peroxide in the non-host pathogen combinations perhaps play an important role in inhibiting or killing the pathogen early after inoculation. These resulted in a matching with the results which indicated that hydrogen peroxide has a key role in resistance to leaf rust (*Puccinia triticina*) in several Egyptian and other wheat resistant cultivars (Hafez *et al*., 2009).

Table (1): Results of inoculated plants with compatible and incompatible pathogens

Plants	Compatible pathogen (host)	Result of infection	Incompatible pathogen (non-host)	Result of infection
Tomato	Tomato powdery mildew	S	Tobacco powdery mildew	R
Datura	Tobacco mosaic virus (TMV)	S	*Papaya ringspot virus* (PRSV)	R
Tobacco	Tobacco powdery mildew	S	Cucumber powdery mildew	R
Cucumber	Cucumber powdery mildew	S	*Tobacco mosaic virus* (TMV)	R
Squash	-	-	Tomato powdery mildew	R
Chenopodium	-	-	PRSV	R

S = susceptible; R = resistant.

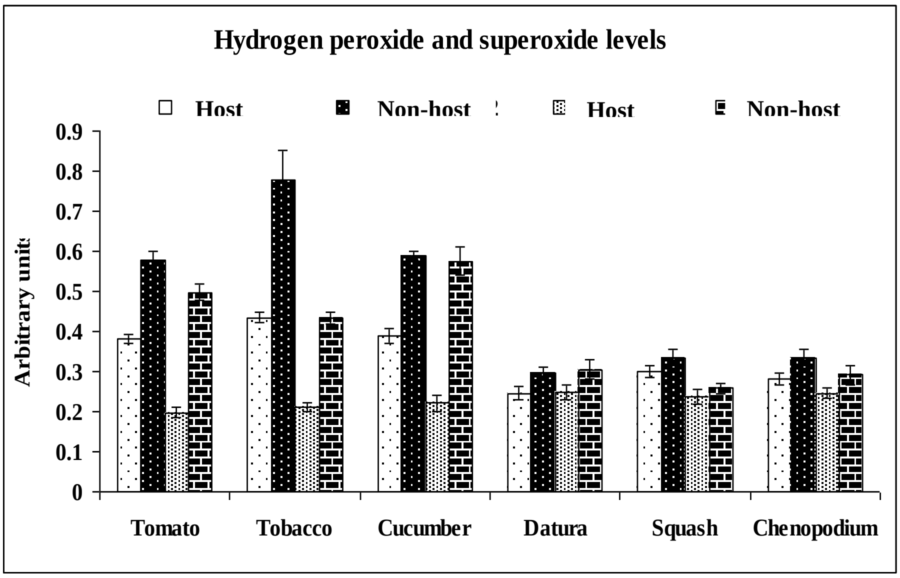

Fig. (1): Levels of superoxide ($O_2^{.-}$) 24 hours after inoculation and hydrogen peroxide (H_2O_2) 36 hours after inoculation in host and non-host / pathogen combinations. Host H_2O_2: level of hydrogen peroxide in tomato, tobacco, cucumber, datura, squash or Chenopodium leaves inoculated with the compatible pathogens. Non-host H_2O_2: level of hydrogen peroxide in inoculated leaves with incompatible pathogens. Host $O_2^{.-}$: level of superoxide in inoculated leaves compatible pathogens. Non-host $O_2^{.-}$: level of superoxide in inoculated leaves with incompatible pathogens.

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Corresponding author: Hafez, Y.M.
E-mail: yasser.abdelgwad@agr.kfs.edu.eg

دور مشتقات الأكسجين الحرة في المقاومة الغير عائلة لمسببات الأمراض النباتية

ياسر محمد حافظ[1]، خالد عبدالدايم عبدالعال[1]، نصر الدين عبدالصمد علي البغدادي[2] ،وأيمن فيصل عمر[1]

1- قسم النبات الزراعي (شعبة أمراض النبات) - كلية الزراعة – جامعة كفر الشيخ – 33516 - كفر الشيج – مصر

2- قسم الوراثة - كلية الزراعة – جامعة كفر الشيخ – 33516 - كفر الشيج – مصر

معظم النباتات في الظروف الطبيعية تكون مقاومة لأغلب مسببات أمراض النبات الغير متوافقة (فيروس- فطر- بكتيريا). هذه الظاهرة تسمى المقاومة الغير عائلة (الغير مضيفة). هذا النوع من المقاومة مهم جدا ولكن لم تجرى بحوث كافية لشرح ميكانيكية هذه المقاومة.

عندما تمت عدوى العديد من العوائل الغير عائلة بمسببات مرضية غير متوافقة فإن مستوى فوق اكسيد الهيدروجين والسوبراكسايد إرتفع إرتفاعا معنويا مبكرا بعد العدوى. تم إثبات هذه الظاهرة في نباتات الطماطم والداتورا والدخان والخيار والكوسة والزربيح والتى أعديت بالمسببات المرضية الغير متوافقة وهى فطر البياض الدقيقى فى الدخان وفيروس التبقع الحلقي فى البابا ظ وفطر البياض الدقيقى فى الخيار وفيروس تبرقش أوراق الدخان وفطر البياض الدقيقى فى الطماطم وفيروس التبقع الحلقي فى البابا ظ على التوالى.

ومن الجدير بالذكر انه عندما أعديت بعض من العوائل السابق ذكرها بالمسببات المرضية المتوافقة لها فإن مستوى فوق اكسيد الهيدروجين والسوبراكسايد لم يرتفع أو يتراكم معنويا.

ويمكن القول بأن مشتقات الأكسجين الحرة وعلى وجه الخصوص فوق اكسيد الهيدروجين والسوبراكسايد ربما تلعب دورا حيويا فى تثبيط او قتل مسببات الأمراض النباتية مبكرا فى النباتات الغير عائلة. ونوصي بمزيد من الإهتمام نحو إستخدام فوق اكسيد الهيدروجين والسوبراكسايد سواء بطرق مباشرة أو بمركبات تنتج أو تستحث إنتاج مشتقات الأكسجين الحرة لمقاومة مسببات أمراض النبات.