www.czasopisma.pan.pl PAN www.journals.pan.pl
POLSKA AKADEMIA NAUK

Journal of Plant Protection Research ISSN 1427-4345

RAPID COMMUNICATION

Effects of bacterial populations, temperature and exogenous hydrogen peroxide on the induction of the hypersensitive response in *Nicotiana tabacum* against *Xanthomonas perforans*

Ali Safaie Farahani, Seyyed Mohsen Taghavi*

Department of Plant Protection, College of Agriculture, Shiraz University, Shiraz, Iran

Vol. 57, No. 2: 201–204, 2017

DOI: 10.1515/jppr-2017-0019

Received: March 12, 2017
Accepted: May 24, 2017

*Corresponding address:
mtaghavi@shirazu.ac.ir

Abstract

The objective of this study was to investigate the effects of inoculum concentration, plant post-inoculation incubation temperature and exogenous hydrogen peroxide (H_2O_2) on the induction of the hypersensitive response (HR) in *Nicotiana tabacum* against *Xanthomonas perforans*. Inoculation of leaves with *X. perforans* at a concentration of 10^8 CFU · ml^{-1} and incubation of plants at 30°C resulted in the strongest HR elicitation. Furthermore, an exogenous supply of H_2O_2 accelerated *X. perforans*-induced HR, whereas *in planta* H_2O_2 removal by application of catalase led to a delay in HR development. Our data suggest that H_2O_2 has an important role in HR of *N. tabacum* against *X. perforans*.

Key words: catalase, hydrogen peroxide, hypersensitive response, *Nicotiana tabacum*, *Xanthomonas perforans*

Plants are constantly exposed to various biotic and abiotic stresses during their life cycle. Among biotic challenges, diseases caused by fungi, bacteria, viruses and nematodes result in serious yield losses worldwide. In general, only a few pathogens have the ability to cause disease in a certain plant species. The resistance exhibited by an entire plant species to all genetic variants of a non-adapted pathogen species is referred to as non-host resistance. It is a durable and broad-spectrum resistance against numerous pathogens (Fan and Doerner 2012; Senthil-Kumar and Mysore 2013). Non-host resistance usually leads to the induction of the hypersensitive response (HR) at the infection site (Senthil-Kumar and Mysore 2013). The HR as the induction of quick necrotic lesions within 24–48 h after inoculation of tobacco leaves by incompatible plant pathogenic bacteria was first reported almost 50 years ago (Klement *et al.* 1964). Cell death rapidly encloses the site of infection and makes it almost impossible to develop biotrophic pathogens. One of the earliest responses of plants to pathogen invasion is the production of reactive oxygen species (ROS) including singlet oxygen (O_2), superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^•$) (Bolwell and Daudi 2009). Reactive oxygen species can restrict the pathogen through various mechanisms such as a direct effect on the invading pathogen (Levine *et al.* 1994), strengthening cell walls via oxidative cross-linking (Bradley *et al.* 1992), induction of systemic acquired resistance (Alvarez *et al.* 1998) and HR (Lamb and Dixon 1997). Accumulation of ROS in several non-host pathosystems such as barley/*Blumeria graminis* f. sp. *tritici* (Hückelhoven *et al.* 2001), cowpea/*Erysiphe cichoracearum* (Mellersh *et al.* 2002) and pepper/*Blumeria graminis* f. sp. *tritici* (Hao *et al.* 2011) has been reported. On the other hand, plants employ scavenging enzymes such as superoxide dismutase, ascorbate peroxidase and catalase for ROS detoxification (Mittler *et al.* 2004). The induction of scavenging enzymes in a number of non-host interactions such as pepper/*Xanthomonas campestris* pv. *campestris* (Kwak *et al.* 2009), broad bean/*Puccinia striiformis* f. sp. *tritici* (Cheng *et al.* 2012), bean/*X. hortorum* pv. *pelargonii* (Safaie Farahani and Taghavi 2015) and mung bean/*X. hortorum* pv. *pelargonii* (Safaie Farahani and Taghavi 2016) has been demonstrated. *Xanthomonas perforans* is the causal agent of bacterial

Table 1. The percentage of tobacco leaves showing hypersensitive response (HR) symptoms in different concentrations of bacterial inoculum and temperatures at 48 hpi (hours post-inoculation)

Temperature [°C]	Bacterial population		
	10^6 CFU · ml^{-1}	10^7 CFU · ml^{-1}	10^8 CFU · ml^{-1}
20	0±0	17±2	50±4
25	25±1	50±5	83±4
30	42±2	67±5	100±0

Table 2. The percentage of tobacco leaves showing hypersensitive response (HR) symptoms after exogenous application of H_2O_2 at different time intervals

Time intervals [hpi]	Treatment			
	control	H_2O_2 at 4/6 hpi	H_2O_2 at 6/8 hpi	H_2O_2 at 8/10 hpi
12	33±2	73±3	60±6	40±2
24	80±4	100±0	100±0	100±0

hpi – hours post-inoculation

spot of tomato, an important disease worldwide (Jones *et al.* 2004). Optimum experimental situations and the role of ROS in non-host resistance of *Nicotiana tabacum* against *X. perforans* remain unclear. The goal of this study was to examine the role of temperature, bacterial populations and exogenous hydrogen peroxide in *X. perforans*-induced HR on tobacco leaves.

Xanthomonas perforans Tom801 (Osdaghi *et al.* 2016) was used in this study. To determine the role of bacterial populations and plant post-inoculation incubation temperature during *X. perforans*-induced HR, the inoculum at three concentrations (10^6 CFU · ml^{-1}; 10^7 CFU · ml^{-1} or 10^8 CFU · ml^{-1}) of bacterial suspension was separately infiltrated in fully expanded leaves of eight-week-old *N. tabacum* plants by needleless syringes. Following infiltration, the plants were incubated at 20°C, 25°C or 30°C. The percentage of leaves showing HR symptoms were recorded at 48 hours post inoculation (hpi). Twelve leaves from three plants were used for each treatment. To investigate the effects of H_2O_2 exogenous application on *X. perforans*-induced HR, the pathogen inoculum at a concentration of 10^8 CFU · ml^{-1} was infiltrated in fully expanded leaves. Afterwards, H_2O_2 at a concentration of 0.1 mM was sprayed twice (to maintain the high concentration of H_2O_2) to the inoculated leaf area at 4/6, 6/8 and 8/10 hpi. Control plants were treated with water instead of H_2O_2. The plants were incubated at 30°C. Necrosis symptoms were checked at 12 and 24 hpi. Fifteen leaves from four plants were used for each treatment. To remove *in planta* H_2O_2, an exogenous catalase was used. First, a bacterial suspension at a concentration of 10^8 CFU · ml^{-1} was infiltrated in fully expanded leaves. In the next step, the catalase at a concentration of 2,000 U · ml^{-1} was infiltrated twice to the inoculated leaf area at 4/6, 6/8 and 8/10 hpi. The plants were incubated at 30°C. Hypersensitive response symptoms were recorded at 24 and 48 hpi. Fifteen leaves from four plants were used for each treatment.

The optimal inoculum concentration and plant post-inoculation incubation temperature for *X. perforans*-induced HR were determined in this study. A high concentration of bacterial inoculum (10^8 CFU · · ml^{-1}) led to more induction of the HR than lower concentrations (10^6 CFU · ml^{-1} and 10^7 CFU · ml^{-1}). Additionally, the plants that were incubated at 30°C showed more induction of the HR than those which were kept at 20°C and 25°C. Interaction of bacterial populations and temperature was also effective in HR induction. The greatest induction of the HR was found at 10^8 CFU · · ml^{-1}/30°C. On the other hand, no HR symptoms were observed at 10^6 CFU · ml^{-1}/20°C (Table 1). There are several reports indicating different effects of inoculum concentration and plant post-inoculation incubation temperature on HR induction and non-host resistance (Jahnen and Hahlbrock 1988; Budde and Ullrich 2000; Li *et al.* 2015). This suggests that the role of these factors might depend on plant species, pathogen and their interaction. However, how the bacterial populations and temperature affect *X. perforans*-induced HR is still worth studying. Exogenous application of H_2O_2 led to faster and greater induction of the HR than the control. Furthermore, earlier exogenous H_2O_2 application (4/6 hpi) resulted in more HR elicitation than other H_2O_2 treatments (6/8 hpi and 8/10 hpi) at 12 hpi. There was no difference in terms of HR symptoms between the

Table 3. The percentage of tobacco leaves showing hypersensitive response (HR) symptoms after exogenous application of catalase at different time intervals

Time intervals [hpi]	Treatment			
	control	catalase at 4/6 hpi	catalase at 6/8 hpi	catalase at 8/10 hpi
24	80±7	40±4	53±3	67±6
48	100±0	53±1	73±6	93±4

hpi – hours post-inoculation

three H_2O_2 treatments at 24 hpi (Table 2). The leaves treated with catalase showed fewer HR symptoms than the control at 24 and 48 hpi. In addition, earlier catalase application (4/6 hpi) led to a greater reduction in HR induction than other catalase treatments (6/8 hpi and 8/10 hpi) at 24 and 48 hpi (Table 3). Our results are in agreement with a previous study by Li *et al.* (2015) that showed that an exogenous supply of H_2O_2 accelerates *Xanthomonas oryzae* pv. *oryzae*-induced HR in *N. benthamiana*. A link between ROS and HR during non-host resistance has been reported in lettuce-*Pseudomonas syringae* pv. *phaseolicola* (Bestwick *et al.* 1997) and tobacco-*Xanthomonas campestris* pv. *vesicatoria* pathosystems (Zurbriggen *et al.* 2009). Yoda *et al.* (2003) revealed that HR induction in tobacco plants inoculated with tobacco mosaic virus is through hydrogen peroxide produced by polyamine degradation. In conclusion, the results of this study demonstrated that HR induction in *N. tabacum* against *X. perforans* is affected by the inoculum concentration and the temperature of plant incubation post-inoculation. Additionally, faster and stronger HR elicitation by exogenous application of H_2O_2 confirmed the role of ROS in HR development. Reduction and delay in HR induction after exogenously supplied catalase also indicate the correlation between ROS accumulation and HR development.

References

Alvarez M.E., Pennell R.I., Meijer P.J., Ishikawa A., Dixon R.A., Lamb C. 1998. Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. Cell 92 (6): 773–784. DOI: 10.1016/S0092-8674-(00)81405-1

Bestwick C.S., Brown I.R., Bennett M., Mansfield J.W. 1997. Localization of hydrogen peroxide accumulation during the hypersensitive reaction of lettuce cells to *Pseudomonas syringae* pv. *phaseolicola*. Plant Cell 9 (2): 209–221. DOI: 10.1105/tpc.9.2.209

Bolwell G.P., Daudi A. 2009. Reactive oxygen species in plant–pathogen interactions. p. 113–133. In: "Reactive Oxygen Species in Plant Signaling (Signaling and Communication in Plants)" (L.A. del Río, A. Puppo, eds.). Springer-Verlag, Berlin Heidelberg, Germany, 246 pp.

Bradley D.J., Kjellbom P., Lamb C.J. 1992. Elicitor- and wound--induced oxidative cross-linking of a proline-rich plant cell wall protein: a novel, rapid defense response. Cell 70 (1): 21–30. DOI: 10.1016/0092-8674(92)90530-P

Budde I.P., Ullrich M.S. 2000. Interactions of *Pseudomonas syringae* pv. *glycinea* with host and nonhost plants in relation to temperature and phytotoxin synthesis. Molecular Plant-Microbe Interaction 13 (9): 951–961. DOI: 10.1094/MPMI.2000.13.9.951

Cheng Y., Zhang H., Yao J., Wang X., Xu J., Han Q., Wei G., Huang L., Kang Z. 2012. Characterization of non-host resistance in broad bean to the wheat stripe rust pathogen.BMC Plant Biology 12 (1): 96. DOI: 10.1186/1471-2229-12-96.

Fan J., Doerner P. 2012. Genetic and molecular basis of non-host disease resistance: complex, yes; silver bullet, no. Current Opinion in Plant Biology 15 (4): 400–406. DOI: 10.1016/j.pbi.2012.03.001

Hao X., Yu K., Ma Q., Song X., Li H., Wang M. 2011. Histochemical studies on the accumulation of H_2O_2 and hypersensitive cell death in the non-host resistance of pepper against *Blumeria graminis* f. sp. *tritici*. Physiological and Molecular Plant Pathology 76 (2): 104–111. DOI: 10.1016/j.pmpp.2011.07.003

Hückelhoven R., Dechert C., Kogel K.H. 2001. Non-host resistance of barley is associated with a hydrogen peroxide burst at sites of attempted penetration by wheat powdery mildew fungus. Molecular Plant Pathology 2 (4): 199–205. DOI: 10.1046/j.1464-6722.2001.00067.x.

Jahnen W., Hahlbrock K. 1988. Cellular localization of nonhost resistance reactions of parsley (*Petroselinum crispum*) to fungal infection. Planta 173 (2): 197–204. DOI: 10.1007/BF00403011.

Jones J.B., Lacy G.H., Bouzar H., Stall R.E., Schaad N.W. 2004. Reclassification of the xanthomonads associated with bacterial spot disease of tomato and pepper. Systematic and Applied Microbiology 27 (6): 755–762. DOI: 10.1078/0723202042369884

Klement Z., Farkas G.L., Lovrekovich L. 1964. Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. Phytopathology 54: 474–477.

Kwak Y.S., Han K.S., Lee J.H., Lee K.H., Chung W.S., Mysore K.S., Kwon Y.S., Kim H.K., Bae D.W. 2009. Different oxidative burst pattern occur during host and non-host resistance responses triggered by *Xanthomonas campestris* in pepper. Journal of Plant Biotechnology 36: 244–254.

Lamb C., Dixon R.A. 1997. The oxidative burst in plant disease resistance. Annual Review of Plant Physiology 48: 251–275. DOI: 10.1146/annurev.arplant.48.1.251

Levine A., Tenhaken R., Dixon R., Lamb C. 1994. H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell 79 (4): 583–593. DOI: 10.1016/0092--8674(94)90544-4

Li W., Xu Y.P., Yang J., Chen G.Y., Cai X.Z. 2015. Hydrogen peroxide is indispensable to *Xanthomonas oryzae* pv. *oryzae*-induced hypersensitive response and nonhost resistance in *Nicotiana benthamiana*. Australasian Plant Pathology 44: 611–617. DOI 10.1007/s13313-015-0376-1

Mellersh D.G., Foulds I.V., Higgins V.J., Heath M.C. 2002. H_2O_2 plays different roles in determining penetration failure in three diverse plant-fungal interactions. The Plant Journal 29 (3): 257–268. DOI: 10.1046/j.0960-7412.2001.01215.x

Mittler R., Vanderauwera S., Gollery M., Van Breusegem F. 2004. Reactive oxygen gene network of plants. Trends in Plant Science 9 (10): 490–498. DOI: 10.1016/j.tplants.2004.08.009

Osdaghi E., Taghavi S.M., Hamzehzarghani H., Fazliarab A., Lamichhane J.R. 2016. Monitoring the occurrence of tomato bacterial spot and range of the causal agent *Xanthomonas perforans* in Iran. Plant Pathology. DOI: 10.1111/ppa.12642.

Safaie Farahani A., Taghavi S.M. 2015. Expression profiling of malate dehydrogenase, superoxide dismutase and polygalacturonase-inhibiting protein in common bean in response to host and non-host pathogens. Journal of Plant Pathology 97 (3): 491–495. DOI: 10.4454/JPP.V97I3.030

Safaie Farahani A., Taghavi M. 2016. Changes of antioxidant enzymes of mung bean [*Vigna radiata* (L.) R Wilczek] in response to host and non-host bacterial pathogens. Journal of Plant Protection Research 56 (1): 95–99. DOI: 10.1515/jppr-2016-0016

Senthil-Kumar M., Mysore K.S. 2013. Non-host resistance against bacterial pathogens: retrospectives and prospects. Annual Review of Phytopathology 51: 407–427. DOI: 10.1146/annurev-phyto-082712-102319.

Yoda H., Yamaguchi Y., San H. 2003. Induction of hypersensitive cell death by hydrogen peroxide produced through polyamine degradation in tobacco plants. Plant Physiology 132: 1973–1981. DOI: 10.1104/pp.103.024737

Zurbriggen M.D., Carrillo N., Tognetti V.B., Melzer M., Peisker M., Hause B., Hajirezaei M.R. 2009. Chloroplast-generated reactive oxygen species play a major role in localized cell death during the non-host interaction between tobacco and *Xanthomonas campestris* pv. *vesicatoria*. Plant Journal 60 (6): 962–973. DOI: 10.1111/j.1365-313X.2009.04010.x.