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Effect of *Ralstonia solanacearum* and *Meloidogyne javanica* on tomato plant antioxidant activity

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Abstract

Meloidogyne javanica and *Ralstonia solanacearum* are the highly specialized soil-born plant parasites with economic importance causing root-knot and bacterial wilt diseases in tomatoes, respectively. The occurrence and intensity of the bacterial wilt escalated in the presence of root-knot nematodes and *R. solanacearum* concurrently detected in different vegetable crops. Sampling and preparation of leaf extract were done to investigate the activity of catalase (CAT), superoxide dismutase (SOD), and peroxidase (POX) enzymes at 24, 48, 72, and 120 hours post-inoculation (hpi) of tomato plants with *R. solanacearum* and *M. javanica*. The enzyme activity was measured at each time interval. The CAT and SOD enzymes exhibited maximum activity levels at 120 and 48 hpi in the nematode treatment, respectively. Meanwhile, the levels of POX enzyme peaked at 48 and 72 hpi in the nematode and nematode-bacterium treatments, respectively. Pathogen stress eventually led to a decrease in the SOD and POX enzymes 120 hours after inoculation and a significant increase in CAT during nematode-bacterium treatment. The results revealed apparent enzyme activity variations in tomato plants infected with both pathogens at different time intervals after inoculation.

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Introduction

The tomato (*Lycopersicon esculentum* L.) is a perennial plant that is usually grown as an annual and belongs to the family *Solanaceae*. In Iran, there are different climatic zones for tomato cultivation and fresh tomato is produced in all seasons. Considering the high nutritional and economic value of this product, it is important to study the limiting factors that affect tomato growth and find appropriate solutions to increase plant yield. Bacterial wilt and the root-knot nematode are among the limiting factors of tomato production worldwide (Wicker et al., 2007). Brown rot or bacterial wilt caused by *Ralstonia solanacearum* is a major threat to tomato cultivation in tropical agriculture (Khokhani et al., 2017). *R. solanacearum* has a broad host range with more than 200 species from 50 plant families and attacks a variety of economically important crops, including potato, tomato, chili, eggplant and non-solanaceous plants such as groundnut (Plener et al., 2010). The economic damage caused by bacterial wilt depends on the host, plant variety, weather conditions, soil type and harvesting method (Genin & Denny, 2012; Schell, 2000). This phytopathogen can reduce the yield of agricultural products by 20-60% (Wang et al., 2023). Root-knot nematodes play an important role in crop yield reduction (Chen & Robert, 2003; Wesemael et al., 2011). Root-knot nematodes are the most important group of plant parasitic nematodes that have a significant economic impact on various agricultural products. *Meloidogyne* species cause a 5% decline in agricultural yields worldwide and pose a significant challenge to the cultivation of adequate food in developing countries (Kiewnick et al., 2009). Root-knot nematodes interact with *R. solanacearum* through several mechanisms, including the formation of wounds that facilitate the penetration of the bacterial pathogens into the plant, the induction of physiological changes in the host leading to increased susceptibility, the disruption of host resistance and the alteration of root microflora (Siddiqui et al., 2012). In the mutual interactions of parasitism, defense mechanisms have

evolved in plants, including enzymatic and non-enzymatic mechanisms (Singh et al., 2021). Production of defense enzymes is one of the host's resistance reactions against plant pathogens. The most rapid defense response after pathogen recognition is the production of reactive oxygen species (ROS), leading to oxidative bursts. ROS play a role in resistance to bacterial diseases and may also directly cause their death (Mittler, 2017). Meanwhile, most bacteria have mechanisms to protect themselves. Bacterial pathogens can tolerate the toxicity of reactive oxygen species (Kapoor et al., 2019). In the present study, the potential of the enzyme activity of peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) was investigated in tomato plants infected with *R. solanacearum* and *M. javanica* alone and in combination.

Material and Methods

Nematode preparation

The galled roots of tomato and cucumber plants were collected from greenhouses at Boyer-Ahmad County, Iran, and a single egg mass of the root-knot nematode, *M. javanica*, was cultured on tomato seedlings (cv. Early-Urbana) in the greenhouse. The nematode species was identified by examining the morphological characteristics of the female perennial pattern. A 0.5% solution of NaOCl (Sodium hypochlorite) was used to extract the nematode eggs from the galled roots, following the method described by Hussey and Barker (1973). The number of eggs was determined using a hand counter.

Ralstonia solanacearum preparation

R. solanacearum isolate Rs130, previously isolated from tomato in Fars Province (Izadiyan & Taghavi, 2011), was used in this study. A pure and fresh bacterial culture was obtained by growing the bacterial suspension on a nutrient sucrose agar (NSA) culture medium. The culture was incubated at 27°C for 48 h. The bacterium was then collected and mixed with sterile distilled water. The concentration was adjusted to 107 CFU/ml.

Greenhouse studies

The study was conducted to investigate the enzyme activities in tomato plants infected with *R. solanacearum* and *M. javanica* in a controlled greenhouse environment at a temperature of 27 ± 4°C and a 16:8 hour light/dark photoperiod. Plastic pots with a diameter of 15 cm were used, each filled with 1.5 kg of a mixed soil (one part cow manure, one part steam-sterilized sandy loam soil and two parts sand). Tomato (cv. Early-Urbana) seeds were planted in pots and inoculated with the nematode and bacterium four weeks later. Plants were then arranged in a completely randomized design with three replicates. Treatments included plants inoculated with *R. solanacearum* and *M. javaniva* simultaneously (RM); inoculated with *R. solanacearum* (Rs); inoculated with *M. javanica* (Mj) and with distilled water as the control.

Determination of enzymes activities

Leaf samples (500 mg) were ground and mixed thoroughly with 1.25 mL of 100 mM phosphate buffer (pH 7.0) containing 1% polyvinyl-poly pyrrolidone (PVPP). Subsequently, the samples were centrifuged (12000 g, 4 ◦C, 20 min) and the same supernatant was exploited to detect SOD, CAT and POX. The amount of SOD was determined using a modified version of the method developed by Patykowski and Urbanek (2003). The reaction mixture consisted of 1.5 ml of 0.05 M sodium phosphate buffer (pH = 7.8), 0.3 ml of 130 mM methionine, 0.3 ml of 750 µM nitro blue tetrazolium (NBT), 0.3 ml of 0.1 mM EDTA-Na2, 0.3 ml of 20 µM riboflavin, 0.01 ml of enzyme extract, 0.01 ml of 4% (w/v) polyvinyl polypyrrolidone (PVPP), and 0.28 ml of deionized water. The reaction was initiated by exposing the tubes to a 20 W fluorescent lamp at a distance of 30 cm for 10 min. After covering the samples with a black cloth for another 10 min, the reduction activity of NBT was quantified as increased absorbance at 560 nm/h g of fresh weight (Patykowski & Urbanek, 2003). Spectrophotometric measurements of the oxidation products by guaiacol at 475 nm were performed to monitor the peroxidase activity during purification, (Reuveni, 1995). The reaction mixture consisted of 2 ml, containing 20 µl of 200 mM guaiacol, 40 µg protein extract and citrate-phosphate buffer (pH = 5.4). Ten µl H2O2 (30% v/v) was added and measurements at 475 nm were taken at six time points 10 s apart. One unit of peroxidase activity was defined as the amount of enzyme catalyzing the oxidation of 1 µmol of guaiacol per minute per milligram of protein at 475 nm. For the evaluation of catalase activity, the reaction mixture consisted of 3 ml of 50 mM sodium phosphate buffer (pH = 7), 30 µg of protein-containing apoplectic extract, and 30 ml of H2O2. The consumption of H2O2 was measured using a spectrophotometer at 240 nm (Nikoo et al., 2014). The activity of the enzymes was determined at 24-hour intervals over a period of one week.

Results

Peroxidase activity

The results of the analysis of variance showed that the effect of *R. solanacearum* or *M. javanica* individually or in combination on peroxidase activity was statistically significant at the 1% level (Table 1). In the treated plants, the induction of peroxidase was faster in the Mj treatment 48 hours after inoculation than in the other treatments. However, on the third day (72 hours) after inoculation, the peroxidase concentration was significantly higher in the RM treatment than in the Rs and Mj treatments (Fig. 1).

Table 1. Analysis of variance of the activity level of the peroxidase in healthy and *Ralstonia solanacearum* and *Meloidogyne javanica* inoculated tomato cv. Early-Urbana plants.

Source	DF	SS	Mean Square	F Value	Pr > F
Time	4	0.00008908	0.00002227	275.75	<.0001
Nematode	1	0.00001723	0.00001723	213.35	<.0001
Bacterium	1	0.00000231	0.00000231	28.6	<.0001
Time*Nematode	3	0.00003052	0.00001017	125.98	<.0001
Time*Bacterium	3	0.00015704	0.00005235	648.14	<.0001
Nematode*Bacterium	1	0.00000431	0.00000431	53.33	<.0001
Time*Nematode*Bacterium	3	0.00006105	0.00002035	251.98	<.0001
Error	34	0.00000275	0.00000008		
Total	50	0.00036868			

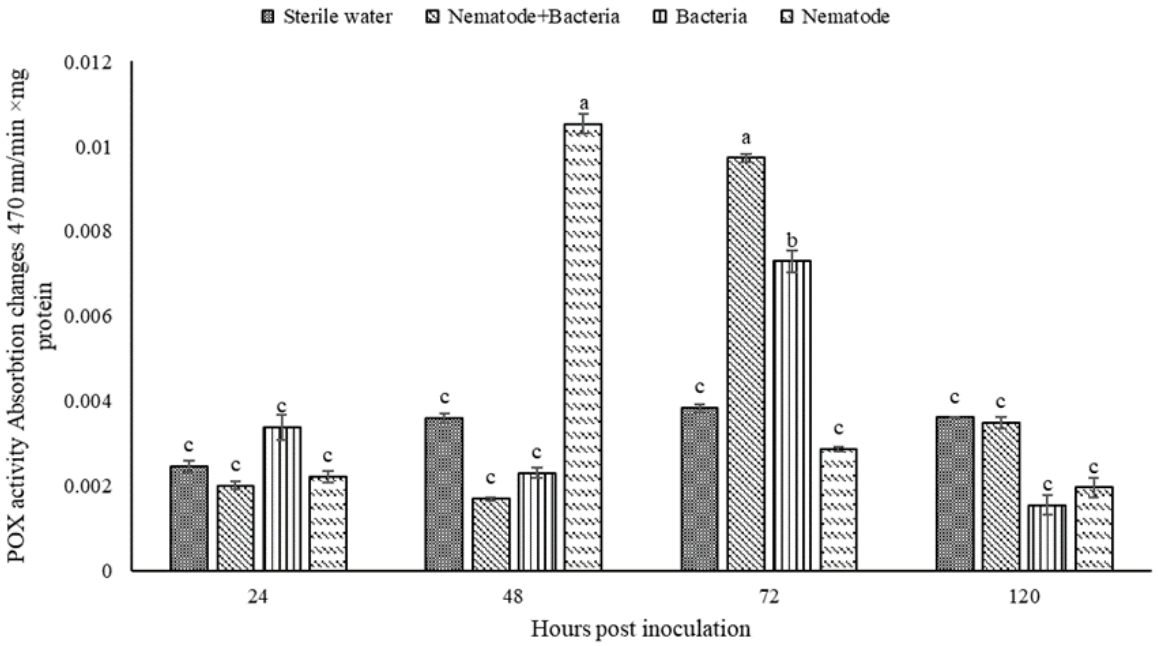

Fig. 1. Effect of *Meloidogyne javanica* and *Ralstonia solanacearum* on the specific activities of peroxidase in inoculated tomato cv. Early-Urbana seedlings 24 hours intervals after inoculation.

Catalase activity

The results of the analysis of variance indicated that the effect of *R. solanacearum* or *M. javanica* individually or in combination on catalase activity was significant at the 1% level (Table 2). The plants inoculated with the bacterium and the nematode (RM) showed CAT activities at all time points. The catalase activity in the leaves increased significantly 120 hours after inoculation, in the Mj treatment compared with the other treatments. There was an

increase in CAT activity in inoculated plants with two pathogens (*RM*) at 72 hours of infection (Fig. 2).

Table 2. Analysis of variance of the activity level of the catalase in healthy and *Ralstonia solanacearum* and *Meloidogyne javanica* inoculated tomato cv. Early-Urbana plants.

Source	DF	SS	Mean Square	F Value	Pr > F
Time	4	0.00028875	0.00007219	277.67	<.0001
Nematode	1	0.00020638	0.00020638	793.83	<.0001
Bacterium	1	0.00006986	0.00006986	268.72	<.0001
Time*Nematode	3	0.0003023	0.00010077	387.6	<.0001
Time*Bacterium	3	0.00087504	0.00029168	1121.95	<.0001
Nematode*Bacterium	1	0.00050251	0.00050251	1932.89	<.0001
Time*Nematode*Bacterium	3	0.00035311	0.00017655	679.11	<.0001
Error	34	0.00000832	0.00000026		
Total	50	118.3665094			

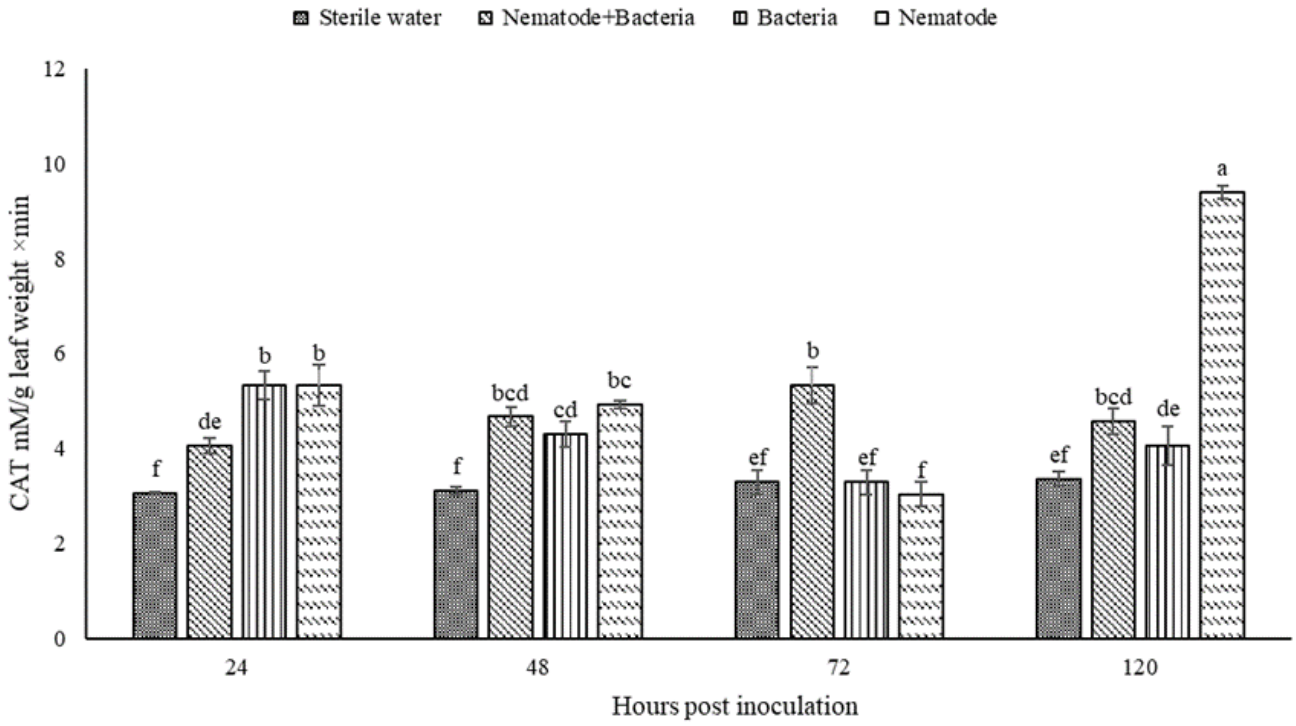

Fig. 2. Effect of *Meloidogyne javanica* and *Ralstonia solanacearum* on the specific activities of catalase in inoculated tomato cv. Early-Urbana seedlings 24 hours intervals after inoculation.

SOD activity

The results of the analysis of variance showed that the effect of *R. solanacearum* or *M. javanica* individually or in combination on superoxide dismutase activity was statistically significant at the 1% level (Table 3). The SOD activity was highest in the Mj treatment at 48 hours after inoculation. The activity of the SOD enzyme increased significantly from 24 hpi to 48 hpi in Mj treatment and then decline until 120 hpi. There was very little change in SOD activity after 72 h of inoculation in all treatments compared with the control. There was an increase in SOD activity in the RM treatment compared with the other treatments at 24 h of infection (Fig. 3).

Table 3. Analysis of variance of the activity level of the superoxide dismutase in healthy and *Ralstonia solanacearum* and *Meloidogyne javanica* inoculated tomato cv. Early-Urbana plants.

Source	DF	SS	Mean Square	F Value	Pr > F
Time	4	0.00028875	0.00007219	277.67	<.0001
Nematode	1	0.00020638	0.00020638	793.83	<.0001
Bacterium	1	0.00006986	0.00006986	268.72	<.0001
Time*Nematode	3	0.0003023	0.00010077	387.6	<.0001
Time*Bacterium	3	0.00087504	0.00029168	1121.95	<.0001
Nematode*Bacterium	1	0.00050251	0.00050251	1932.89	<.0001
Time*Nematode*Bacterium	3	0.00035311	0.00017655	679.11	<.0001
Error	34	0.00000832	0.00000026		
Total	50	118.3665094			

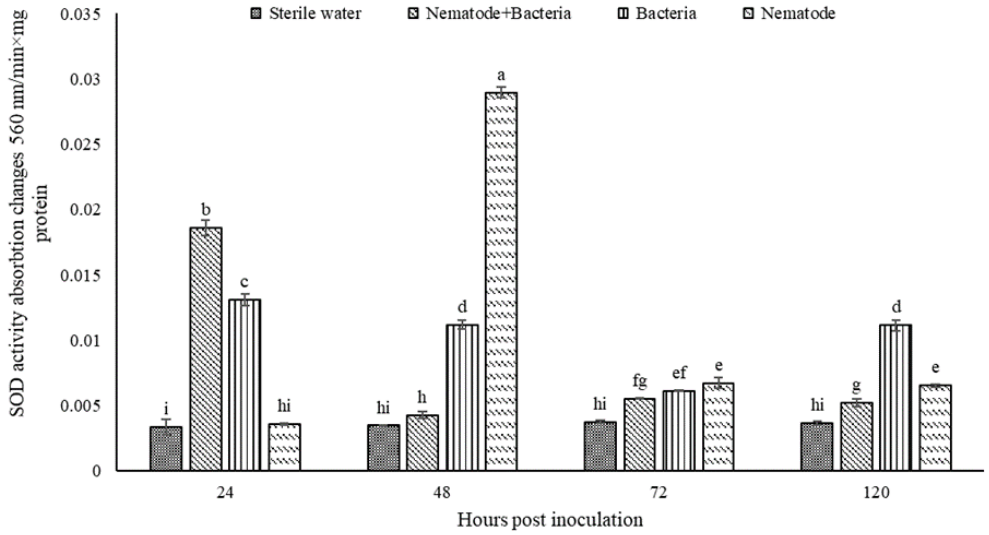

Fig. 3. Effect of *Meloidogyne javanica* and *Ralstonia solanacearum* on the specific activities of catalase in inoculated tomato cv. Early-Urbana seedlings 24 hours intervals after inoculation.

Discussion

This study focused on antioxidant activity in the leaves of tomato plants inoculated with the root-knot nematodes *M. javanica* and the bacterium *R. solanacearum*. Tomatoes can face challenges from various pathogens, including root-knot nematodes (*M. javanica*) and *R. solanacearum*, a pathogen that causes bacterial wilt in many plants. Antioxidant enzymes play a crucial role in the plant's defense mechanisms against such stresses (Mittler, 2017). These enzymes help in scavenging reactive oxygen species (ROS), which are generated during stress conditions (Kayani & Mukhtar, 2018). Both nematode and bacterium infections can trigger the expression of defense-related genes, leading to the synthesis of various defense proteins, including antioxidant enzymes (Kapoor et al., 2019). The activation of a diverse range of defense responses in plants is linked to disease resistance, which effectively hinders or stops infection during specific stages of the interaction between the host and pathogen (Feng et al., 2015). Upon infection, the host triggers a series of pathogen-inducible enzymes that play a crucial role in defending against phytopathogens (Pilon et al., 2011). In the present study, the induction of peroxidase (POX) occurred more rapidly in the Mj treatment at 48 hours after inoculation than in the other treatments. However, at 72 hours after inoculation, the concentration of POX was significantly higher in the RM treatment than in the Rs or Mj treatments. When a root-knot nematode infects a root, it causes minimal damage as it moves between

cells. However, an excessive production of reactive oxygen species (ROS) can be observed as soon as the nematode penetrates the root at the J2 stage. Some evidence has shown that a barrier forms in cells with high peroxidase activity at the beginning of giant cell formation, which later disappears. This observation suggests that peroxidases are produced by plants as an initial reaction against nematode invasion. The increasing trend of peroxidase (POX) in response to *M. javanica* in tomato plants may be connected to cell wall lignifications. This increased structural rigidity of plant tissues helps to hinder nematode penetration. It is well-documented that POX activity plays a role in the final stages of cell wall lignifications (Quiroga et al., 2000). The severity and incidence of bacterial wilt increased when root-knot nematodes were detected in conjunction with *R. solanacearum* in numerous vegetable varieties (Tariq-Khan et al., 2017). Increased POX activity has been reported in different plants such as tomato (Ramamoorthy et al., 2002), rice (Nandakumar et al., 2001; Nithya et al., 2019) and cucumber (Chen et al., 2000) under the influence of various pathogens. Root-knot nematodes enable the entry and establishment of pathogenic fungi and bacteria (Powell, 1971). POX levels in plants can increase in response to biotic stress, indicating that POX activities are involved in plant defense against pathogens (Kartashova et al., 2000). In tomato, peroxidase is one of the enzymes that catalyse the steps in the lignification pathways (Ramamoorthy et al., 2002; Nithya et al., 2019). Lignin enhances plant cell wall strength, boosting plant immunity against pathogen enzymes and providing a protective barrier against physical penetration (Nicholson & Hammerschmidt, 1992). The current investigation highlights the rapid reaction of POX in tomato plants infected with *R. solanacearum* and *M. javanica*, suggesting the potential involvement of this enzyme in the defense against pathogenic invasion. Our findings are similar to the results of Vanitha & Umesha (2008) on tomato infected with *R. solanacearum* and Chittoor et al. (1997) on rice infected with *Xanthomonas oryzae* pv. *oryzae*. In our study, there was an increase in catalase (CAT) activity in inoculated plants with two pathogens (RM treatment) at 72 h of infection. CAT is an antioxidative enzyme involved in oxidative bursts generated transiently in plant-microbe interactions. The use of enzyme CAT is involved in the regulation of H2O2 levels in plant tissues (Mandal et al., 2011). Catalase has already been reported to be induced systemically in potato infected with M. incognita (Niebel et al., 1995) and the possible role of catalase inhibition in tomato resistance to root-knot nematodes was emphasized (Molinari, 2001). The enzyme catalase acts as a specific guaiacol peroxidase, shielding cells from the harmful effects of H2O2 (Ben Amor et al., 2005). It was observed that sugar beet genotypes infected with M. incognita displayed a marked increase in catalase activity (Korayem et al., 2012). Furthermore, plants treated with *M. javanica* also exhibited elevated catalase activity. Moreover, Kesba & El-Beltagi (2012) found that CAT activity increased by 46.97%, 57.97%, and 68.25% in superior, superior/freedom, and freedom grape rootstocks infected with M. incognita compared with healthy plants. In the presents study, SOD activity was highest in the Mj treatment at 48 h after inoculation. SOD activity increased significantly from 24 to 48 hpi in the Mj treatment and then declined until 120 hpi. There was very little change in SOD activity after 72 h of inoculation in all treatments compared with uninoculated plants. An increase in SOD activity was observed in the RM treatment compared with other treatments at 24 hours after infection. In recent years, some studies have reported that SODs can protect plants against biotic and abiotic stresses, such as cold, salinity, drought, abscisic acid and ethylene (Asensio et al., 2012; Wang et al., 2004).

Conclusion

The results of this study indicate different responses of enzyme activities in tomato plants cv. Early-Urbana subjected to different treatments and different times after inoculation with *M. javanica* and *R. solanacearum*. The specific patterns observed in the activities of peroxidase, superoxide dismutase, and catalase activities highlight the dynamic nature of plant defense mechanisms in response to different pathogens. These results contribute to a better understanding of plant immune responses under different stress conditions.

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Conflict of interest

The authors declare that there are no conflicts of interest present.

CRediT author statement

M. Panahi: Performed the experiments. **R. Rezaei**: Supervised the study, reviewing and edited the manuscript. **H. Charehgani**: Analyzed the experimental data.

References

Asensio, A. C., Gil-Monreal, M., Pires, L., Gogorcena, Y., Aparicio-Tejo, P. M., & Moran, J. F. (2012). Two Fe-superoxide dismutase families respond differently to stress and senescence in legumes. *Journal of Plant Physiology, 169*, 1253–1260.

Ben Amor, N., Ben Hameda, K., Debeza, A., Grignonb, C., & Abdelly, C. (2005). Physiological and antioxidant responses of the perennial halophyte *Crithmum maritimum* to salinity. *Plant Science, 168*, 889-899.

Chen, C., Belanger, R. R., Benhamou, N., & Paulitz, T. C. (2000). Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. *Physiological and Molecular Plant Pathology, 56(1)*, 13-23.

Chen, P., & Robert, P. A. (2003). Virulence in *Meloidogyne hapla* differentiated by resistance in common bean. *Nematology, 5*, 39-47.

Chittoor, J. M., Leach, H. E., & White, F. F. (1997). Differential induction of peroxidase gene family during infection of rice by *Xanthomonas oryzae* pv. *oryzae*. *Molecular Plant-Microbe Interactions Journal, 10*, 861-871.

Feng, X., Lai, Z., Lin, Y., Lai, G., & Lian, C. (2015). Genome-wide identification and characterization of the superoxide dismutase gene family in *Musa acuminata* cv. Tianbaojiao (AAA group). *BMC Genomics, 16*, 823.

Genin, S. (2010). Molecular traits controlling host range and adaptation to plants in *Ralstonia solanacearum*. *New Phytologist, 187*, 920–928.

Genin, S., & Denny, T. P. (2012). Pathogenomics of the *Ralstonia solanacearum* species complex. *Annual Review of Phytopathology, 50*, 67-89.

Hussey, R. S., & Barker, K. R. (1973). Comparison of methods of collecting inoculate of *Meloidogyne* spp. including a new technique. *Plant Disease Reporter, 57*, 1025–1028.

Izadiyan, M., & Taghavi, M. (2011). Diversity of Iranian isolates of *Ralstonia solanacearum*. *Phytopathologia Mediterranea*, 50, 236-244.

Kapoor, D., Singh, S., Kumar, V., Romero, R., Prasad, R., & Singh, J. (2019). Antioxidant enzymes regulation in plants in reference to reactive oxygen species (ROS) and reactive nitrogen species (RNS). *Plant Gene, 19*, 100182.

Kartashova, E. R., Rudenskaya, G. N., & Yurina, E. V. (2000) Plant Peroxidase Polyfunctionality and Their Application in Practice. *Journal of Agricultural Biology, 1*, 63-70.

Kayani, M. Z., & Mukhtar, T. (2018). Reproductivity of *Meloidogyne incognita* on fifteen cucumber cultivars. *Pakistan Journal of Zooogy, 50*, 1717-1722.

Kesba, H. H., & El-Beltagi, H. S. (2012). Biochemical changes in grape rootstocks resulted from humic acid treatments in relation to nematode infection. *Asian Pacific Journal of Tropical Biomedicine, 2*, 287-293.

Khokhani, D., Lowe-Power, T. M., Tran, T. M. & Allen, C. (2017). A single regulator mediates strategic switching between attachment/spread and growth/virulence in the plant pathogen *Ralstonia solanacearum*. *MBio*, *8*, e00895–17.

Kiewnick, S., Dessimoz, M., & Franck, L. (2009). Effects of the Mi-1 and the N root-knot nematode-resistance gene on infection and reproduction of *Meloidogyne enterolobii* on tomato and pepper cultivars. *Journal of Nematology, 41(2)*, 134-139.

Korayem, A. M., El-Bassiouny, H. M. S., El-Monem, A. A. A., & Mohamed, M. M. M. (2012). Physiological and biochemical changes in different sugar beet genotypes infected with root-knot nematode. *Acta Physiologiae Plantarum, 34*, 1847-1861.

Mandal, S., Das, R. K., & Mishra, S. (2011). Differential occurrence of oxidative burst and antioxidative mechanism in compatible and incompatible interactions of tomato and *Ralstonia solanacearum*. *Plant Physiology and Biochemistry, 49*, 117-123.

Mittler, R. (2017). ROS are good. *Trends in Plant Science, 22(1)*, 11–19.

Molinari, S. (2001). Inhibition of H2O2-degrading enzymes in the response of *Mi*-bearing tomato to root-knot nematodes and salicylic acid treatment. *Nematologia Mediterranea, 29*, 235-239.

Nandakumar, R., Babu, S., Viswanathan, R., Raguchander, T., & Samiyappan, R. (2001). Induction of systemic resistance in rice against sheath blight disease by *Pseudomonas fluorescens*. *Soil Biology and Biochemistry, 33(4-5)*, 603- 612.

Nicholson, R. L., & Hammerschmidt, R. (1992). Phenolic compounds and their role in disease resistance. *Annual Review of Phytopathology, 30*, 369-389.

Niebel, A., Heungens, K., Barthels, N., Inzé D., Van Montagu, M., & Gheysen, G. (1995). Characterization of a pathogen-induced potato

catalase and its systemic expression upon nematode and bacterial infection. *Molecular Plant Microbe Interaction, 8 (3),* 371–378.

Nikoo, F. S., Sahebani, N., Aminian, H., Mokhtarnejad, L., & Ghaderi, R. (2014). Induction of systemic resistance and defense-related enzymes in tomato plants using *Pseudomonas fluorescens* CHAO and salicylic acid against root-knot nematode *M. javanica*. *Journal of Plant Protion Research, 54*, 383–389.

Nithya, K., Shanmugaiah, V., Balasubramanian, N., & Gomathinayagam, S. (2019). Plant defence related enzymes in rice (*Oryzae sativa* L.,) induced by *Pseudomonas* sp. vsmku2. *Journal of Pure and Applied Microbiology, 13(3),* 1307-1315.

Patykowski, J., & Urbanek, H. (2003). Activity of enzymes related to H2O2 generation and metabolism in leaf apoplastic fraction of tomato leaves infected with *Botrytis cinerea*. *Journal of Phytopathology, 151(3),* 153–161.

Pilon, M., Ravet, K., & Tapken, W. (2011). The biogenesis and physiological function of chloroplast superoxide dismutases. *Biochimica et Biophysica Acta, 1807(8),* 989–998.

Plener, L., Manfredi, P., Valls, M., & Genin, S. (2010) PrhG, a transcriptional regulator responding to growth conditions, is involved in the control of the type III secretion system regulon in *Ralstonia solanacearum*. *Journal of Bacteriology*, *192*, 1011–1019.

Powell, N. T. (1971). Interaction of plant parasitic nematodes with other disease-causing agents. In B. M. Zuckerman, W. F. Mai & R.A. Rohde (eds.), *Plant parasitic nematodes*. (pp. 119-136), Academic Press Inc., London.

Quiroga, M., Guerrero, C., Botella, M. A., BarceloÂ Amaya, I, Medina, M. I., Alonso, F. J., de Forchetti, S. M., Tigier, H., & Valpuesta, V. (2000). A tomato peroxidase involved in the synthesis of lignin and suberin. *Plant Physiology, 122*, 119-1127.

Ramamoorthy, V., Raguchander, T., & Samiyappan, R. (2002). Induction of defense related proteins in tomato roots treated with *Pseudomonas fluorescens* Pf1 and *Fusarium oxysporum* f. sp. *lycopersici*. *Plant and Soil, 239(1),* 55-68.

Reuveni, R. (1995). Biochemical markers as tools for screening resistance against plant pathogens. p. 21–42. In R. Reuveni (ed.), *Noval Approaches to Integrated Pest Management* (pp. 21-42), CRC Press, Boca Raton, USA.

Schell, M. A. (2000) Control of virulence and pathogenicity genes of *Ralstonia solanacearum* by an elaborate sensory network. *Annual Review of Phytopathology*, *38*, 263–292.

Siddiqui, Z. A., Nesha, R., Singh, N., & Alam, S. (2012). Interactions of plant parasitic nematodes and plant pathogenic bacteria. In D. K. Maheshwari (ed.). *Bacteria in Agrobiology. Plant Probiotics* (pp. 251-267), Springer-Verlag: Berlin Heidelberg.

Singh, Y., Nair A. M., & Verma P. K. (2021). Surviving the odds: From perception to survival of fungal phyto-pathogens under host-generated oxidative burst. *Plant Communications, 2(3),* 100142.

Tariq-Khan, M., Munir, A., Mukhtar, T., Hallmann, J., & Heuer, H. (2017). Distribution of root-knot nematode species and their virulence on vegetables in northern temperate agro-ecosystems of the Pakistani-administered territories of Azad Jammu and Kashmir. *Journal of Plant Disease Protection*, *124*, 201-212.

Vanitha, S. C., & Umesha, S. (2008) Variations in defense related enzyme activities in tomato during the infection with bacterial wilt pathogen. *Journal of Plant Interactions*, *3*, 245-253.

Wang, B., Lüttge, U., & Ratajczak, R. (2004). Specific regulation of SOD isoforms by NaCl and osmotic stress in leaves of the C3, halophyte *Suaeda salsa* L. *Journal of Plant Physiology*, *161*, 285–293.

Wang, Z. J., Luo, W. B., Cheng, S. J., Zhang, H. J., Zong, J., & Zhang, Z. (2023). *Ralstonia solanacearum* - A soil borne hidden enemy of plants: research development in management strategies, their action mechanism and challenges. *Frontiers in Plant Science*, *14*, 1141902.

Wesemael, W. M., Viaene, N., & Moens, M. (2011). Root-knot nematodes (*Meloidogyne* spp.) in Europe. *Nematology, 13,* 3-16.

Wicker, E., Grassart, L., Coranson-Beaudu, R., Mian, D., Guilbaud, C., & Fegan, M. (2007). *Ralstonia solanacearum* strains from Martinique (French West Indies) exhibiting a new pathogenic potential. *Applied and Environmental Microbiology*, *73*, 6790–6801.