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QUANTITATIVE CHANGES IN SUPEROXIDE DISMUTASE, CATALASE AND PEROXIDASE WITH REFERENCE TO RESISTANCE IN TOMATO TO *MELOIDOGYNE INCOGNITA*

S.P. RAJASEKHAR, A.K. GANGULY AND S.C. SWAIN

Division of Nematology, Indian Agricultural Research Institute, New Delhi- 110012

Abstract: Investigations were carried out to establish a link between the activities of superoxide dismutase (SOD), peroxidase (PO), catalase and accumulation of superoxide radicals (O_2^0) in tomato- *Meloidogyne incognita* interactions. Time course studies on SOD revealed that the activity of the enzyme was of lower magnitude as compared to the healthy counterpart during entire post infectional period in the resistant cultivar. There was a corresponding increase in the production of superoxide radicals in the nematode inoculated resistant host. These observations were suggestive of the fact that in resistant host, nematode infection leads to inactivation of SOD which results in production of O_2, consequently affecting the biology of the invading pathogen adversely. The increase in the enzyme activity in susceptible host was found to be maximum at 72 hrs after inoculation. There was also an increase in PO activity in inoculated Pusa Ruby, a susceptible host but was of lower order as compared to resistant host. The trend of catalase activity followed the same pattern as that of SOD activity in both the cultivars.

Key words: Superoxide dismutase, peroxidase, catalase, *Meloidogyne incognita*, tomato.

Considerable contributions on the role of different enzymes in relation to hypersensitive reaction of host against invading pathogen have been made (Halliwell, 1978a; Ganguly & Dasgupta, 1979, 1980; Premachandran & Dasgupta, 1983; Raja & Dasgupta, 1986; Ganguly & Dasgupta, 1988; Sirohi & Dasgupta, 1993). Further these investigations and other reports available in the literature do indicate one significant fact that a flux of superoxide radicals (O_2.) extending to cell membranes could indeed be lethal towards pathogenic microorganisms (Babior, 1978; Klebanoff, 1980). Zacheo & Bleve-Zacheo (1988) documented that nematode infection leads to the production of superoxide anions and in susceptible cultivars these were scavenged by the enhanced superoxide dismutase (SOD) activity. In the resistant cultivars, the relative inactivity of SOD produce increased superoxide anions, which may be the possible cause of cell necrosis and the hypersensitive reaction. Nonetheless, very few workers have made an attempt to document a comparative account of plant nematode interaction on the basis of SOD activity and production of superoxide radical involving incompatible and compatible hosts in sequential event (Ganguly & Dasgupta, 1988; Zacheo & Bleve-Zacheo, 1988). In conjugation of these events, relevant is the phenomenon which provides an avenue to explore information on the fate of hydrogen peroxide in relation to catalase and peroxidase (PO) activity. It is because hydrogen peroxide has been shown to exhibit cytotoxicity (Halliwell & Gutteridge, 1989) and its removal is accomplished by the action of catalase and peroxidase. The sequential role of these three enzymes namely, SOD, catalase and peroxidase, on the production of superoxide radicals and peroxidation in compatible and incompatible hosts during post infection

period has scantly been studied. In order to understand the mechanism of hypersensitive reaction as a defence mechanism against invading pathogenic phytophagous nematodes, the role of these three enzymes in a system comprising compatible and incompatible hosts and nematode pathogen, during the initial stage of post infectional period was examined. The objective of the present investigation was quantitative estimation of superoxide dismutase, peroxidase and catalase in compatible (Pusa Ruby) and incompatible (Mangla) tomato cultivars against root-knot nematode, *Meloidogyne incognita* Race 1.

MATERIALS AND METHODS

M. incognita Race 1 propagated originally from a single eggmass progeny, was reared and multiplied on greenhouse grown tomato cultivar Pusa Ruby. Experiment confirmed tomato cultivar Mangla as resistant to the population of *M. incognita* Race 1 was used in this study. Two weeks after transplanting, each seedling was inoculated with 5000 freshly emerged, axenized infective J_2 of *M. incognita*. A set of each tomato cultivar was kept uninoculated to serve as control.

Preparation of crude extract

Plants of each cultivar, both uninoculated and inoculated were harvested at predetermined intervals (24, 48, 72 and 168 hrs). Five to 10g of shoots and roots from all treatments were chopped into small pieces under equal volume of prechilled 0.05 M sodium potassium phosphate buffer, pH 4.7 containing 0.1 per cent ascorbic acid, 0.1 per cent cystein hydrochloric acid and 17 per cent sucrose, and homogenized in ceramic pestle and mortar cooled in ice. The slurry was strained through a sterilized four layered cheese cloth, centrifuged at 16,000 g for 20 min. The supernatant solution was stored at 12°C as and when required. The supernatant solution was partially purified through Sephadex G-25 chromatography and fractions were monitored spectrophotometrically at 620 nm for protein at 460 nm for peroxidase (Shannon *et al.*, 1971) and at 240 nm for catalase (Chance & Maehly, 1955). The fractions showing the enzymic activity were pooled together and dialysed against sucrose. The resultant amount was served as crude for further analysis.

Assay of Superoxide dismutase

The enzymatic activity of SOD was assayed photochemically according to the method of Ravindranath & Fridovich (1975). The reaction mixture consisted of 15mM potassium phosphate/ buffer, pH 7.8 containing 100 mM EDTA; 100 µl of 1 mM nitroblue tetrazolium (NBT); 150 µl of 100 mM methionine; 30 µl of riboflavin and 30 µl of enzyme extract, water was added to make the volume 3 ml and then exposed to fluorescent light for 10 min. Under assay conditions, the absorbance at 560 nm increased linearly with the time of illumination. The light intensity was such that it caused an increase of absorbance of 0.05 per min. One unit of enzyme is defined as that amount which caused 50 per cent inhibition of NBT reduction, under experimental conditions.

Catalase

The enzymic activity of catalase was assayed by measuring the initial rate of disappearance of hydrogen by the method of Chance & Maehly (1955). The 3 ml reaction mixture contained 50 mM phosphate buffer, pH 7.0, 15 mM hydrogen peroxide and 25 µl enzymes extract. The decrease in hydrogen peroxide was measured as a decline in the absorbance at 240 nm on spectrophotometer. A unit of enzyme is expressed as the amount of catalase which converts one µm of hydrogen peroxide per minute, under the experimental conditions.

Peroxidase

The enzymes activity of peroxidase was determined following the method of Shannon *et al.* (1971). The reaction mixture consisted of 2.8 ml O-dianisidine-buffer solution (0.5% O- dianisidine in 0.6 M sodium acetate buffer, pH 5.4), and 100 µl of enzyme extract. The reaction was started by the addition of H_2O_2 and the rate of substrate oxidation was measured at 460 nm on a spectrophotometer using 1 cm light path. The reaction rate was found to be linear during the first one minute. A unit of enzyme is defined as the amount of enzyme which will increase absorbance of 1 A^0 per min. per ml under the assay conditions at 30°C.

Assay of superoxide

The detection of superoxide was based on its ability to reduce NBT to formazan (Doke, 1983). The reaction mixture consisted of 3 ml of 0.01 potassium phosphate buffer, pH7.8; 1 mM EDTA; 20 µm NADPH; 0.05% of NBT and 0.2g (fresh weight) of plant material. The complete reaction mixture without NBT was maintained under vaccum for 5 min. and was then exposed to the atmosphere (Oxygen). The NBT was added and 30 min. later the reaction mixture was heated at 85°C for 15 min. The reduced NBT was calculated from O.D. at 580 nm., the reducing activity of NBT was expressed as increase of O.D. of 580 per h per g of dry weight.

RESULTS

Sequential development of superoxide dismutase activity

The enzymes activity increased with senescence in root and shoot of both the cultivars (Table 1 & 2). In the susceptible

TABLE 1: Changes in peroxidase (PO), superoxide dismutase (SOD) and catalase in tomato roots (Pusa Ruby and Mangla) inoculated with *M. incognita* Race -1.

Hours after inoculation	Per cent increase (+) or decrease (-) over control (uninoculated)					
	Pusa Ruby			Mangla		
	PO	SOD	Catalase	PO	SOD	Catalase
24	+ 29.95	+7.00	+18.76	+ 68.99	-3.30	-22.91
[illegible]	+76.24	+62.98	+26.67	+50.31	-11.55	-33.03
72	+96.38	+90.06	+60.00	+37.86	-19.21	-31.84
168	+68.44	+78.02	+53.75	+21.20	-6.49	-16.68

TABLE 2. Changes in peroxidase (PO), superoxide dismutase (SOD) and catalase in tomato roots (Pusa Ruby and Mangla) inoculated with *M. incognita* Race -1.

Hours after inoculation	Per cent increase (+) or decrease (-) over control (uninoculated)					
	Pusa Ruby			Mangla		
	PO	SOD	Catalase	PO	SOD	Catalase
24	+ 8.81	+4.04	+8.96	+ 41.94	-1.41	-17.87
48	+16.04	+36.95	+34.64	+25.93	-5.01	-23.44
72	+31.27	+58.76	+49.22	+14.74	-16.08	-24.89
168	+39.97	+46.97	+50.84	+9.35	-9.58	-17.20

cultivar it increased due to the infection of *M. incognita* Race 1. But in the resistant cultivar, enzyme activity was observed to be decreasing after inoculation. At 72 HAI (hours after inoculation) the maximum increase in susceptible cultivar (90.06 per cent in root and 58.76 per cent in shoots) and maximum decrease in resistant cultivar (-19.21 per cent in root and - 16.08 per cent in shoot) over their respective controls was observed with regard to the SOD activity.

Sequential development of catalase activity

The activity of catalase was observed to increase with senescence in roots and shoots of both compatible and incompatible cultivars (Table 1 and 2). In the susceptible cultivar, the increase in catalase activity was recorded during post inoculation period. The maximum increase in shoot (50.84 per cent) was recorded at 168 HAI. The activity of enzymes was decreased in the resistant cultivar with inoculation. The maximum decrease (-33.03 per cent) was recorded at 48 HAI in root. But, in shoots, maximum decrease (-24.89 per cent) was observed at 72 HAI.

Sequential development of peroxidase

An increase in the enzyme activity was observed in roots and shoots of both the cultivars with senescence and inoculation (Table 1 and 2). The rate of increase in the enzyme activity was in ascending order in susceptible cultivar. In contrast, resistant cultivar exhibited a decreasing trend for the same observation. In the susceptible cultivar, the maximum increase in roots (97.38 per cent) and shoots (39.97 per cent) was observed at 72 and 168 HAI, respectively. In the resistant cultivar, the maximum increase in roots (68.99 per cent) and in shoots (41.94 per cent) over their corresponding control was recorded at 24 HAI. In the course of time, the increase came to the minimum levels in root (21.20 per cent) and shoot (9.35 per cent) at 168 HAI.

Time course changes in superoxides

An increase in the production of superoxides with senescence was observed in both the cultivars (Table 3). There was a constant decrease of free radicals in roots of inoculated susceptible cultivar. The maximum decrease (-20.69 per cent) was recorded at 168 HAI. The level of superoxides was increased in resistant cultivar with inoculation. The maximum increase (21.89 per cent) was observed at 72 HAI in the roots of resistant cultivar.

TABLE 3. Changes in the activity of superoxide radicals (OD 580/h/dry weight)* of tomato roots (cultivars Pusa Ruby and Mangla) inoculated with *M. incognita* Race 1.

Hours after inoculation	Susceptible cultivar (Pusa Ruby)			Resistant cultivar (Mangla)		
	Uninoculated	Inoculated	Per cent increase (+) or decrease (-) over control	Uninoculated	Inoculated	Per cent increase (+) or decrease (-) over control
24	109.21±3.55	103.73±4.03	-5.02	141.47±8.22	146.92±6.24	+3.85
48	116.14±5.16	102.53±5.98	-11.71	149.06±3.82	178.50±5.25	+19.75
72	123.42±4.07	99.72±5.96	-19.20	156.27±6.73	190.48±3.76	+21.89
168	140.81±9.01	111.67±7.25	-20.69	190.01±4.08	214.26±8.07	+12.76

* Each figure represents a mean value with ± S.Em of assays performed in triplicate

DISCUSSION

This investigation showed the incompatible interaction of Mangla cultivar to *M. incognita* was characterized by the comparatively lower magnitude of the SOD activity and a corresponding elevated level of superoxide radicals. The elevated level of peroxidase activity was noted in the incompatible plant reaction in the initial and early stage of post- infectional period within 24 HAI. The catalase activity was found to be less in an inoculated incompatible tomato plant.

Superoxide radicals have been implicated to have toxic effect on the biomembranes of the living entity (Huang, 1985). Scavenging of these radicals is accomplished through the enzyme superoxide dismutase. Apparently, this had adverse effect on the development of the nematode. In contrast, enhanced superoxide dismutase activities leading to complete scavenging of superoxide radical in a compatible host, tomato cultivar Pusa Ruby was observed. In our opinion the high peroxidase activity observed within the 24 HAI in the incompatible host can be considered a part of general activation of the host cell to impart resistant responses on cell necrosis by next 48 h of post infectional period. Seemingly, a strong correlation exists between early induction of peroxidase activity and hypersensitive response of incompatible Mangla cultivar to *M. incognita* infection. It is unlikely that the infecting nematode juveniles and developing populations within the root contributed to enhanced level of the enzyme as contaminant. Since earlier reports and our observations indicate that it is not possible to detect the enzyme activity with the inoculum level used. It was maintained that senescence of PO isozymes could be one of the reasons to explain why a host plant becomes susceptible to a pathogen like nematode (Farkas & Stahmann, 1966; Ganguly & Dasgupta, 1979). Likewise, this investigation also noted senescence of PO in the susceptible plant 48 HAI, a phenomenon which was lacking in the healthy counterpart.

There is a striking similarity between the catalase activity and to that of superoxide dismutase. It has already been found that catalase reduce and effectively inactivate hydrogen peroxide in eukaryotic cells (Halliwell, 1978 b; Halliwell & Gutteridge, 1989). The reduced catalase activity in incompatible host as compared to susceptible tomato cultivar, Pusa Ruby could indicate enhanced level of reactive hydrogen peroxide which ultimately could lead to the destruction of pathogen or could induce pathogen related gene expression.

One of the experiments of investigation was to seek information whether or not nematode infection induces systemic biochemical changes in the shoot. The experimental results of this investigation in terms of SOD, PO and catalase were almost identical to that of root samples. In conclusion, this investigation provided a link between host incompatibility and the enzymic activities of superoxide dismutase, peroxidase and catalase in *M. incognita*- tomato system.

REFERENCES

Babior, B.M. (1978). Oxygen dependent microbial killing by phagocytes.*New England J. Medicine.* **298**: 658-668.

Chance, B. & Maehly, A.C. (1955). Assay of catalases and peroxidase. In: Methods in Enzymology (S.P. Colowick and Kapplan, eds.). Academic Press, New York **2**: 764-775.

Doke, N. (1983). Involvement of superoxide anion generation in the hypersensitive response of potato tuber tissues to infection with an incompatible race of *Phytophthora infestans* and to the hyphal wall components. *Physiol.Plant Pathol.* **23**: 345-357.

Farkas, G.L. & Stahmann, M.A. (1966). On the nature of changes in peroxidase isozymes in bean leaves infected by southern bean moasaic virus. *Phytopathology* **56**: 669-677.

Ganguly, A.K. & Dasgupta, D.R. (1979). Sequential development of peroxidase (E.C. 1.11.1.7) and IAA oxidase activities in relation to resistant and susceptible responses in tomatoes to the root-knot nematode, *Meloidogyne incognita. Indian J. Nematol.* **9**: 189-204.

Ganguly, A.K. & Dasgupta, D.R. (1980). Purification and some properties of peroxidase and IAA oxidase from resistant and susceptible varieties of *Lycopersicon esculentum* infested with root-knot nematode, *Meloidogyne incognita, Indian J. Nematol.* **10**: 189-204.

Ganguly, A.K. & Dasgupta, D.R. (1988). Superoxide dismutase (E.C. 1.15.1.1) activity in resistant and susceptible cowpea cultivars inoculated with root-knot nematode, *Meloidogyne incognita. Indian J. Nematol.* **18**: 322-325.

Halliwell, B. (1978a). Biochemical mechanism accounting for the toxic action of oxygen on living organism. The key role of superoxide dismutase. *Cell. Biol. Nt. Reptr.* **2**: 113-120.

Halliwell, B. (1978b). Lignin synthesis: the generation of hydrogen peroxide and superoxide by horse raddish peroxidase and its stimulation by maganese (II) and phenols. *Planta* **140:** 81-88.

Halliwell, B. & Gutteridge, J.M.C. (1989). Free radicals in Biology and Medicine. 2nd Edn. Oxford University Press, Oxford.

Huang, J.S. (1985). Mechanism of resistance to root-knot nematodes. In: An Advanced Treatise on *Meloidogyne* Vol. 1. edit, by Sasser,J.N. and Carter, C.C pp 165-174, NC, USA.

Klebanoff, S.J. (1980). Oxygen metabolism and toxic properties of phagocytes. *Ann. Inter. Medicine* **93**: 480-489.

Premachandran, D. & Dasgupta, D.R. (1983). Changes in nucliec acids and ribonuclease in tomato plants during infection by the root knot nematode, *Meloidogyne incognita. Revue. Nematol.* **6**: 33-38.

Raja, A. & Dasgupta, D.R. (1986). Enhanced synthesis of messenger RNA in relation to resistance expression in cowpea (*Vigna unguiculata*) infected with the root-knot nematode, *Meloidogyne incognita*. *Revue Nematol.* **9**: 35-43.

Ravindranath, S.D. & Fridovich, I. (1975). Isolation and characterization of a maganese containing superoxide dismutase from yeast. *J.Biol. Chemistry*. **250**: 6107.

Shannon, L.M., Uritani, I. & Imaseki, A. (1971). De novo synthesis of peroxidase isozymes in sweet potato slices. *Plant Physiol.* **47**: 493-498.

Sirohi, A. & Dasgupta, D.R. (1993). Mechanism of resistance in cowpea to the root knot nematode *Meloidogyne incognita* Race 1. 1. Early induction of phenylalanine ammonia lyase (E.C. 4.3.1.5) and chlorogenic acid. *Indian J. Nematol.* **23**: 31-41.

Zacheo, G. & Bleve-Zacheo, T. (1988). Involvement of superoxide dismutase and superoxide radicals in the susceptible and resistant tomato plants to *Meloidogyne incognita* attack. *Physiol. Mol. Plant Pathol.* **32**: 313-322.

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