A wide range of organisms infect wheat, the most important being fungi, virus and plant parasitic nematodes, all affecting yield quality and quantity. Several nematodes have been reported associated with wheat crops but the cereal cyst nematodes (CCN), mainly Heterodera avenae Woll., cause damage of almost $78\ billion worldwide (Barker et al., 1998), including India (Gaur and Pankaj, 2009). Heterodera avenae is a sedentary and highly specialized endoparasite. In India it has been reported from the states of Rajasthan, Haryana, Delhi, Himachal Pradesh, Jammu and Kashmir, Madhya Pradesh, Punjab and Uttar Pradesh (Kaushal et al., 2007). Several options are available for the management of the nematode but the role of tolerance/resistance inducing factors cannot be ignored.

Plants react defensively to pathogen invasion in several ways. Secondary metabolites, non-proteinaceous compounds and proteins are usually synthesized in plants following the invasion of the pathogen. Activation of the oxidative burst is a central component of a highly amplified and integrated signal system, also involving salicylic acid and perturbations of cytosolic Ca^{2+}, which underlies the expression of disease-resistance mechanisms (Lamb and Dixon, 1997). Systemic acquired resistance (SAR) confers quantitative protection against a broad spectrum of microorganisms in a manner comparable to immunization in mammals, although the underlying mechanisms differ. Different mechanisms have been reported to be involved in SAR, including lignification and other structural barriers, pathogenesis-related proteins and their expression, and the signals for SAR including salicylic acid (SA) (Sticher et al., 1997). Foliar applications of benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH) and the microbial protein harpin, applied in various combinations, timings, and rates, showed no effects on microbial biomass, Pseudomonas populations, or N-mineralization potentials over 2 years (Collins et al., 2006). Foliar sprays and soil drenches with DL-β-amino-n-butyric acid (BABA) reduced the number of H. avenae. The enzyme activity increased 5 days after inoculation of H. avenae. The percent increase or decrease of the enzyme activity of LOX, PO, PPO and PAL over the control was in the range 10-270%.

**Key words:** Induced systemic resistance, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, lipoxygenase, *Triticum aestivum*.

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**MATERIALS AND METHODS**

The investigation was conducted during the *rabi* season of the year 2009-2010 in a screen-house.

**Soil sterilization**

Field soil from the Indian Agricultural Research Institute was used for the experiment. The soil was mixed with sand in the ratio of 3:1 and sieved through a 20 mesh sieve to ensure uniformity in particle size. The soil-sand mixture was steam-sterilized at 1.05 kg/cm$^2$ pressure for 4 h and stored in polythene bags. Before
use, it was exposed to sunlight by spreading it over a polythene sheet for aeration. A portion of soil (250 g) was taken for determination of soil pH, and the relative proportions of sand, silt and clay, which were 7.5, 68%, 13% and 19%, respectively. Surface sterilized (with 4% formalin) earthen pots (10 cm diam.) were filled with about 500 cm³ of the steam sterilized soil mixture. Three random samples of the sterilized soil were processed by Cobb’s modified sieving and Baermann funnel technique (Cobb, 1918) and examined to ensure that the sterilized soil was free from nematodes.

Chemicals used
Three chemical elicitors, BABA, JA, and SA, were selected based on their association with SAR in plants observed in previous investigations (Hooft Van Huijstijn et al., 1986; Cohen et al., 1994; Tomoya et al., 1998). They were obtained from Sigma-Aldrich, USA and from CDH, India.

Treatments
Two seeds of the wheat cultivar HD 2329, susceptible to attack by *H. avenae*, were sown in each pot and thinned to one plant per pot seven days after sowing. Pots were regularly watered until the end of the experiment. Plants were treated with elicitors 15 days after sowing (at the 2-leaf stage). The treatments used were *L*-amino butyric acid (BABA) at concentrations of 2000, 4000, 6000 and 8000 µg/ml, jasmonic acid (JA) and salicylic acid (SA) at 25, 50, 100 and 200 µg/ml, all used as foliar sprays, and carbofuran (3G at 2 kg a.i./ha). Untreated inoculated and untreated un inoculated plants were used as controls.

Juveniles of cyst nematode and preparation of inoculum
Cysts of the Jaipur population of *H. avenae* were used for inoculation. The cysts were extracted from soil, put in cavity blocks containing sterile water, incubated at 17-18 °C for 5 days and observed for emergence of second stage juveniles (J₂s). The freshly hatched juveniles were collected in a beaker and the suspension density was estimated by counting those in three 1-ml sub-samples, untreated inoculated and untreated uninoculated plants were used as controls.

Enzyme assay
**Peroxidase (PO)** activity. Weighed wheat root samples (1 g) were homogenized in a pre-cooled pestle and mortar in 1.5 ml of 50 mM Tris-HCl buffer, (pH 7.5) at 4 °C. The homogenate was centrifuged at 18,000 rpm for 20 min. at 5 °C. The supernatant was collected in sterilized 2 ml tubes and stored in a freezer at -20 °C before being used as an enzyme source. The enzyme extract was used for assay of PO.

The quantitative estimation of PO was done according to the method described by Jennings et al. (1969). A 0.5 ml extract was placed into a spectrophotometer cuvette into which 0.5 ml of 1% guaiacol solution and 1.5 ml Tris-HCl buffer (0.05 M, pH 7.5) were added. The reaction was initiated by adding 0.5 ml of 1% hydrogen peroxide and optical density readings were taken at 470 nm.

A blank consisting of 0.5 ml of 1% guaiacol and 2.0 ml of Tris-HCl buffer was used to set the spectrophotometer at 100% absorbance. The unit of enzyme activity was expressed as increase in the absorbance at 480 nm min⁻¹ mg⁻¹ of root.

**Polyphenol oxidase (PPO)** activity. Root samples (1 g) were homogenised in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 16,000 rpm (20861 g) for 15 min at 4 °C. The supernatant was used as an enzyme source. The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µl of the enzyme extract. To start the reaction, 0.01 M catechol was added and the activity was expressed as change in absorbance at 495 nm min⁻¹ g⁻¹ of root (Mayer et al., 1965).

**Phenylalanine ammonia lyase (PAL)** activity. PAL was determined quantitatively at 290 nm spectrophotometrically following the method of Peltonen and Karjalainen (1995). The reaction mixture containing 2.5 ml of 0.2% L-phenylalanine in 50 mM Tris-HCl (pH 8.5) and 0.5 ml of supernatant was incubated for 1 h at 37 °C after which the O.D. was recorded at 290 nm. One unit of enzyme is defined as ng of cinnamic acid produced per min. per mg of root.

**Lipoxygenase (LOX)** activity. To measure LOX activity, 0.4 g of wheat roots was extracted in 0.7 ml of 0.2 M sodium-phosphate buffer (pH 6.5) mixed with 0.1% Triton X 100. The extracts were centrifuged at 15,000 g for 25 min at 4 °C and supernatants were collected and stored at -20 °C until use. Root extract (20 µl) was added to 980 µl of 8 × 10⁻⁴ M linoleic acid (Sigma) as substrate solution (Peever and Higgins, 1989) and the activity was measured spectrophotometrically at 234 nm. The activity was expressed as a change in absorbance at 234 nm per min per gram root (Δ A234 × min⁻¹ × g root⁻¹).

Statistical analysis
The experiment was conducted according to a completely randomised design (CRD) and each treatments was replicated three times. Analysis of variance was performed for all data and the different treatment means were compared with Duncan’s multiple range test.
Fig. 1. Effect of foliar application of the chemical elicitors (concentrations in ppm) jasmonic acid (JA), salicylic acid (SA) and DL-ß-amino-n butyric acid (BABA) on peroxidase (PO) activity in wheat roots, 5 (5 DAI) and 10 (10 DAI) days after nematode inoculation, in comparison with carbofuran (2 kg a.i/ha) and controls (IU = inoculated untreated; UU = uninoculated and untreated). Capital letters for data at 5 DAI and lower case for data 10 DAI. Histograms with the same letter are not significantly different according to Duncan’s multiple range test at P = 0.05.

Fig. 2. Effect of foliar application of the chemical elicitors (concentrations in ppm) jasmonic acid (JA), salicylic acid (SA) and DL-ß-amino-n butyric acid (BABA) on polyphenol oxidase activity in wheat roots, 5 (5 DAI) and 10 (10 DAI) days after nematode inoculation, in comparison with carbofuran (2 kg a.i/ha) and controls (IU = inoculated untreated; UU = uninoculated and untreated). Capital letters for data at 5 DAI and lower case for 10 DAI. Histograms with the same letter are not significantly different according to Duncan’s multiple range test at P = 0.05.
Fig. 3. Effect of foliar application of the chemical elicitors (concentrations in ppm) jasmonic acid (JA), salicylic acid (SA) and DL-β-amino-γ-butyric acid (BABA) on activity of phenylalanine ammonia lyase (PAL) in wheat roots, 5 (5 DAI) and 10 (10 DAI) days after nematode inoculation, in comparison with carbofuran (2 kg a.i/ha) and controls (IU = inoculated untreated; UU = uninoculated and untreated). Capital letters for data at 5 DAI and lower case for 10 DAI. Histograms with the same letter are not significantly different according to Duncan’s multiple range test at P = 0.05.

Fig. 4. Effect of foliar application of chemical elicitors (concentrations in ppm) jasmonic acid (JA), salicylic acid (SA) and DL-β-amino-γ-butyric acid (BABA) on lipoxygenase activity in wheat roots, 5 (5 DAI) and 10 (10 DAI) days after nematode inoculation, in comparison with carbofuran (2 kg a.i/ha) and controls (IU = inoculated untreated; UU = uninoculated and untreated). Capital letters for data at 5 DAI and lower case for 10 DAI. Histograms with the same letter are not significantly different according to Duncan’s multiple range test at P = 0.05.
RESULTS AND DISCUSSION

There was little variation of enzyme activity in the inoculated and carbofuran treated plants. On the other hand, significant increases in enzyme activity were observed following the foliar spray of the three elicitors and this increase in enzyme activity was greater with the increase in concentration of the elicitors, with a few exceptions. Also, it is worth mentioning that the enzyme activity 5 days after inoculation (DAI) was generally significantly greater than that at 10 DAI.

Peroxidase activity (Fig. 1) increased with the increase in concentration of the three elicitors and was greatest in plants treated with BABA at 8000 and 6000 µg/ml (1.93 units/g and 1.88 units/g root, respectively), followed by significantly lesser values for JA at 200 µg/ml (1.77 units/g root) and SA at 200 µg/ml (1.66 units/g root). Also, peroxidase activity was further reduced after a further 5 days. PPO activity was greatest in plants treated with BABA 8000 µg/ml followed by JA 200 µg/ml and the increase over the control for both these treatments was highly significant.

PAL also showed similar changes in its activity, which was greatest (0.35 unit/g root) in plants treated with BABA at 8000 µg/ml, followed by SA at 200 µg/ml and JA at 200 µg/ml. The activity decreased with time and was least in control plants.

In general, lipoxygenase activity increased with the increase in concentration of the elicitor at 5 DAI. It was greatest in plants treated with SA at 200 µg/ml followed by JA at 200 µg/ml and BABA at 8000 µg/ml. Again, the activity decreased with time and was significantly less by 10 DAI.

The increase in enzyme activity was in the order of LOX, PO, PPO and PAL. Oka and Cohen (2001) reported that soil drenches with higher concentrations of BABA inhibited development of adult males and females of *H. avenae*. Several chemical elicitors of induced resistance were tested for their ability to reduce the number of *H. avenae* cysts on wheat. Only BABA was found to be an effective resistance inducer.

The increase in the PO, PPO, PAL and LOX activity in susceptible wheat roots could be correlated with resistance responses observed following the application of the three elicitors at their highest concentration. It is known that peroxidase is involved in creating chemical and physical barriers against invading pathogens via cell wall lignification and protein cross-linking, generation of cytotoxic compounds, and/or oxidizing compounds that are important for pathogen metabolism (Kloz et al., 1998). Previous studies demonstrated that cyst nematode infection enhances plant class III peroxidases (PRX), esterase, and superoxide dismutase activity in wheat roots carrying Cre2, Cre5 or Cre7 resistance genes (Andrés et al., 2001; Montes et al., 2004). The greatest response was found in the PRX system with an increase in both cationic and anionic isozymes.

Foliar spray of JA is also capable of increasing LOX activity in plants (Grimes et al., 1992; Rafi et al., 1996), and LOX is also thought to be involved in plant protection and JA synthesis (Sembdner and Parthier, 1993). In oat leaves, greater LOX activity was involved in a resistance reaction to the fungus *Puccinia coronata f. sp. avenae* P. Syd. et Syd. (Yamamoto and Tani, 1986). It has been reported that stimulation of PPO activity was due to the activation of latent enzyme and by the appearance of newly synthesized PPO isozymes during infection (Ganguly and Dasgupta, 1984; Mohanty et al., 1986). Tomato roots infected with *Pratylenchus penetrans* Cobb accumulated chlorogenic acid, which is further oxidized to melanin by PPO and forms a brown patch in the infected tissues (Huang and Rohde, 1973).

Wildermuth et al. (2001) suggested that the phenylpropanoid pathway is responsible for the rapid production of SA associated with local cell death and, in turn, levels of PAL increase with increase in SA concentration in plants. The increase in PAL activity has frequently been reported as a defence reaction of plants to pathogen attack, showing significant increases after infection by pathogens or wounding (Chet, 1993; Seki et al., 1999, Cui et al., 2000; Logemann et al., 2000). Vasyukova et al. (2007) correlated the involvement of PAL and SA with the induction of resistance in tomato plants to the root-knot nematode *M. incognita* (Kofoid et White) Chitw.

The increase in the activity of these enzymes may be attributed to infection of the nematodes along with foliar application of the inducing molecules, according to earlier reports (Ganguly and Dasgupta, 1979; Mohanty et al., 1986; Oka et al., 1997). The greater activity of PO, PPO, PAL and LOX following the foliar application of these elicitors could be correlated with the reduced nematode penetration, final nematode population and fecundity of *H. avenae* (Pokhare et al., 2010). Considering all the plant variables and nematode development during earlier studies (Pokhare et al., 2010), BABA at 8000 µg/ml was the best elicitor, followed by JA at 200 µg/ml and SA at 200 µg/ml, in protecting wheat plants from the cereal cyst nematode.

Resistant plants respond to nematode infection by activating a number of inducible responses that are thought to be disease resistance mechanisms. However, although there are different studies on this subject (Zacheo et al., 1997), very little substantive information exists on resistance genes and biochemical mechanisms occurring during nematode infection. The activation of a mechanism producing active oxygen species, the induction of a peroxidase-catalysing lignification and an antioxidant process have been associated with cell death in resistant plants during nematode feeding site establishment.

The results of this study suggest that PO, PPO, PAL and LOX may be involved in the defence mechanisms of plants, either directly or indirectly, through the production of lignin. It is possible that all of these enzymes are induced in cells surrounding those reacting in a hypersensitive manner.
LITERATURE CITED


Rafi M.M., Zemetra R.S. and Berger P.H., 1996. Jasmonic acid methyl ester-induced protein profile modifications in...


