Ultrastructural changes and oxidative stress markers in wild and cultivar *Sesamum orientale* L. following *Alternaria sesami* (Kawamura) Mohanty and Behera. inoculation

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*Alternaria sesami* causes leaf spot disease in *Sesamum orientale*. Conidium germination, inoculation, penetration and colonization of the pathogen on the plant surfaces were studied using scanning electron microscopy. Electron microscopy analysis revealed multiple germ tubes from conidium that spread in all direction across the leaf surfaces. Penetration in the plant surface occurred, directly through the epidermis or via stomata with or without the appressoria formation. Hyphal penetration continued through the substomata cavity and some of hyphal branches grew in the intercellular space of mesophyll tissue. Hyphal toxin, caused cell and cell wall damages. Changes in different biochemical parameters in the diseased sesame plants (both in wild and cultivar) were compared to control. Transmission electron microscopy showed structural changes in the chloroplast of diseased plants. Isozyme pattern and assays of different enzymes, namely catalase, acid phosphatase and peroxidase expressed varied level of activities. Meanwhile, esterase, polyphenol oxidase and superoxide dismutase in diseased plants showed remarkable levels compared to control. Due to the infection, chlorophyll content, carbohydrates and total soluble protein decreased whereas free amino acid, proline, phenols and disease-related proteins increased in the host plants. Differential SDS-PAGE band profiling of total soluble proteins were also observed in plants due to the infection.

**Keywords:** *Alternaria sesami*, Antioxidant enzymes, Appressoria, Electron microscopy, Isozyme, Protein banding, *Sesamum orientale*

Sesame (*Sesamum orientale* L.), a member of Pedaliaceae is perhaps the oldest oilseed crop known and used by human beings. It is an important annual oilseed crop in the tropics and warmer subtropics. Sesame described as the “Queen of oil seeds” because of its nutraceutical qualities. Sesame seed oil has long shelf life due to the presence of lignans which have remarkable antioxidant function, resisting the oxidation. India is the world leader in sesame production. In India, Sesame is cultivated on an area of 2.18 m.ha with a production of 0.73 million tons annually. It is predominantly grown in Uttar Pradesh, Rajasthan, Orissa, Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka, West Bengal, Bihar and Assam states.

Alternaria leaf spot of sesame, has been identified as a predominant biotic pressure of single origin, that limits seed yield both qualitatively and quantitatively. The resistance mechanism in sesame is obscure. The pathogen can survive between cropping seasons or unfavourable conditions as an infectant in the seeds. Seedlings raised from infected seeds become primary source of inoculum for infection to other plants in the field. Prevention of penetration by pathogens during plant infection is generally dependent on an accurate time course of the pathogen perception by host cells and activation of a signalling cascade triggering a network of coordinated responses. The objective of this investigation is to describe the penetration and infection processes of *A. sesami* on wild and cultivar *S. orientale* using electron microscopy and subsequently, the patho-physiological and biochemical changes that take place in the diseased plant.

**Materials and Methods**

**Collection and isolation of the fungus**—Leaves of sesame (*Sesamum orientale* L.) showing typical symptoms mainly on leaf blades as small, brown, round to irregular spots caused by *Alternaria*...
Alternaria sesami were collected from University of Agricultural Sciences, Bangalore and the fungus isolated by the following technique indicated below.

The infected leaf bits along with some healthy portions were cut into small bits and surface sterilized using 1:1000 mercuric chloride solutions for 30 sec. The bits were washed thoroughly in sterile distilled water for three times to remove traces of mercuric chloride. The molten warm potato dextrose agar (PDA) medium was poured in sterilized petri plates and allowed to solidify. The surface sterilized leaf bits were placed on PDA medium. These plates were incubated at room temperature 28±2 °C and observed periodically for fungal growth. Apparently pure colonies were developed from PDA slants. The slants were incubated at 28±2 °C for sporulation for 10-18 days. Then, such slants containing apparently pure culture were used for further studies.

Total of ten isolates were isolated from different growing areas. The pathogen was identified up to species level based on their cultural and morphological characters. Then the identification was further confirmed from Indian type culture collection and identification, culture supply services, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi.

Wild and Thilarani the cultivar Sesamum orientale were raised from seeds in healthy conditions in a glasshouse. For *in vitro* fungal inoculation studies, mature plants were inoculated with 20 µL of *Alternaria sesami* conidial suspension (1×10^5 conidia mL^-1) from pure culture or 20 µL of water (mock inoculation). The inoculated plants, along with their respective healthy controls, were then maintained at 30 °C in a temperature controlled glasshouse under a photoperiod of 12/12 h (light/dark) and 60% RH. After the development of symptoms in infected plants (after 10 and 14-d of inoculation in the wild and Thilarani plants respectively) the experiment was terminated and the plants harvested for all analysis.

**Scanning and transmission electron microscopy (SEM and TEM)—**Sample of sesame leaf pieces collected after infection were prefixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 6.8, for 2 h at 0-4 °C. After washing with the same buffer, small pieces of leaves were post fixed in 1% OsO₄ at 0-4 °C for 2 h. The material was dehydrated in ethanol followed by propylene oxide and later embedded in Spurr-Epon mixture. Ultrathin sections cut on a Reichert Ultratome were stained with uranyl acetate and lead citrate and examined in a JEOL 1010 transmission electron microscope at 80 kV. The ultrastructure of chloroplasts was studied in mesophyll cells from the middle parts of the fourth leaves from the control and treated plants.

**Analytical and biochemical—**Concentration of chlorophyll in leaves from diseased plants together with the respective healthy controls was determined using a standard protocol. Total phenols, RP-HPLC analysis of phenols, total free amino acids, proline, carbohydrates and total protein were analysed. Polyacrylamide gel electrophoresis (SDS-PAGE) of total soluble protein was conducted using a 12% resolving gel and 5% stacking gel in tris-glycine-SDS buffer following the protocol of Laemmli. Analysis of disease-related proteins was also performed from healthy and diseased plants. The isoenzyme profiles of catalase (CAT), esterase (EST), acid phosphatase (ACP), peroxidase (POX), polyphenol oxidase (PPO) and superoxide dismutase (SOD) were also examined by native PAGE. Activity assays of catalase (CAT), esterase (EST), acid phosphatase (ACP), and peroxidase (POX), polyphenol oxidase (PPO) and superoxide dismutase (SOD) were also evaluated in diseased and healthy plants.

**Statistical analysis—**All the experiments were conducted six times and data presented were average of replicates along with the standard deviation. Database was subjected to an analysis of variance (ANOVA) (STATGRAPHICS Centurion, XV version 15.1.02 year 2006, StatPoint, Inc., USA). Significance between control and treatments were compared at 0.05 and 0.01 probability levels. Discrimination among the means was conducted using Fisher’s least significant difference (LSD) procedure.
Results and Discussion

Scanning electron microscopic analysis—The hyphal network of A. sesami grew extensively over abaxial leaf surface and fill the whole leaf epidermal cell. Conidia of A. sesami were small (6-28 µm) and septate with filiform beaks. Multiple branched germ tubes (length 9-240 µm) were produced from the conidium and grew profusely all over across the leaf surface (Fig. 1). Appressoria are not formed directly on the cuticle or on stomata. Most germ-tubes grew directly into stoma with or without forming an appressorium over the stomata while others showed direct penetration into the host tissue (Fig. 2). Subsequently, the dense germ tubes formed a hyphal mesh on the host mesophyll tissue (Fig. 3) leading to a central darkened area. Conidia were observed primarily on the surface of lesion on mature or senescent leaves and also on wilted twigs (Fig. 4). Some of the hyphal branches were ramifying intercellularly along with other paranchyma tissues. The toxin produced by the fungal hyphae may be the cause for cell damage and cell wall disruption.

Conidium production was highest under moisture or humidity condition but lowest in wet leaves. Conidia germinate quickly if moisture is present and begin to produce toxin even before they penetrate the tissue. Penetration has been consistently associated with formation of appressoria in most species, however, in the present study, penetration occurs either through stomata on the abaxial surface of the leaf with or without the formation appressoria or directly via the epidermal cells.

The mode of infection process of A. sesami observed in the present study was generally similar to that of A. alternata on Minneola tangelo\(^23\). The present results are in consistent with those of Van Den Bery et al\(^24\). Production of multiple germ-tubes that grew randomly across the leaf surface in A. sesami is comparable with that of Alternaria citri. Electron microscopic studies in Cassia infected by Alternaria

Figs 1-4 — (1) Conidium producing several germ-tubes at random points on the conidium body and at the tip of the filiform beak. Bar = 100 µm; (2) Branched germ-tubes passing open stomata without forming appressoria. Bar = 10 µm; (3) Inner surface of the leaf, showing direct penetration of hyphae through the epidermis. Bar = 10 µm and (4) A young conidiophore developing through a stoma of leaf. Bar = 10 µm.
cassiae” has shown appressoria associated with germ tube may have an adhesive function. In the present study, germ tubes and their growth were extremely variable, but this is not unusual because similar response have been reported in cowpea infected with Alternaria. Similarly, SEM observations in sesame showed both direct and indirect penetration with or without the formation of appressoria suggesting that appressoria are not always necessary for infection. The hyphal penetrations were seldom observed prior to necrosis and that the death of mesophyll cells in advance of fungal penetration suggests the action of diffusible fungal toxins by A. sesami. Secondary hyphae produced from primary hyphae penetrate and grew intercellulary. Most of the damages by the Alternaria may be due to the production of cell wall lytic enzymes such as polygalacturonase, pectin lyase, pectin methylestrase, cellulase and toxins - host specific toxins (HST) and non host specific toxins (NHST).

TEM analysis—Ultrastructural changes in the chloroplast of cultivar (Thilarani) include change in the shape with swollen wave like oriented thylakoids separated from the limiting membrane (Fig. 5 a, b and c). The deformities were comparatively pronounced in the cultivar Thilarani than the wild species. Similar changes in chloroplasts accompanied by large grana composed of 40-50 thylakoids were reported in orange rust of Rubus after Gymnoconia peckiana infection; thylakoid undulation and swelling were also described in Mesembryanthemum crystallinum leaves infected by Botrytis cinerea. The configurational changes in the chloroplast may inturn affect the photosynthetic productivity in the plants.

Pigments content—The data presented in Table 1 clearly elucidate that there was a drastic decrease in the amount of photosynthetic pigments in A. sesami infected leaf of the cultivar Thilarani, when compared with the control plants. Thus, there is a positive correlation between the disease severity and chlorophyll level in the infected plants (Table 1). However, there was no significant difference between infected and healthy wild sesame species. The decrease in chl a was more pronounced than chl b, which might affect the photosynthetic efficiency. The data was statistically significant at $P < 0.01$ or $P < 0.05$ levels. The decline in chlorophyll content was supported by the deformity in ultrastructure of chloroplast. The pathogenic fungi may reduce the rate of photosynthesis in the infected leaves by affecting either the chloroplasts or chlorophyll content directly or the photosynthetic enzymes. When a foliar pathogen establishes infection inside host tissues, the chlorophyll content is usually decreased. This is accompanied by yellowing of the infected leaf. Plant Fig. 5 (a-c)—Ultra structural changes like vacuolation, thylakoid deformities in the chloroplast of infected leaves of S. orientale. a. Control, b. wild infected, c. Thilarani infected.
pathogens are known to produce toxic metabolites, which may destroy the chloroplast resulting into decrease of chlorophyll pigments. The decrease in chlorophyll pigments due to foliar infection has been reported in many plant-pathogen interactions\(^{30}\). Gabara \textit{et al.}\(^{28}\) suggested that the decrease of chlorophyll in infected plants may be explained by an inhibition caused by the fungal toxins on photophosphorylation. The significant decrease in chlorophyll a, b and carotenoids of the infected leaves of Thilarani compared to the control healthy plants was in agreement with many studies which reported that the fungal pathogens cause a reduction in chlorophyll concentration to a great extent\(^{31}\). Dan \textit{et al.}\(^{32}\) correlated the decreased amount of chlorophyll a and b with disease intensity by leaf blight in windflower. Increased carotenoid content in the diseased plants protect chlorophylls against oxidative destruction caused by biotic or abiotic stress.

\textbf{Carbohydrate content—}\textit{It is obvious that the A.sesami infection significantly caused a marked decrease in soluble sugars (reducing sugars and sucrose) and insoluble sugars (polysaccharides) in Thilarani compared with the control (Table 2). Decrease in soluble sugars progress with the experiment both in healthy and infected plants (data not shown). Similarly, the polysaccharides decreased in infected leaves but were mostly stable in healthy control leaves over time. Thilarani cultivar had the significant level of decline in soluble and insoluble sugars than wild infected sesame. However, there was no significant difference in these contents between healthy and infected after 10 days of inoculation. Carbohydrates, principally sugars and starch, are the most abundant organic constituents of plants serving as important sources of energy enabling plants to survive through periods of disease, nutrient depletion or drought stress. Infection by pathogenic fungi may lead to substantial changes in the carbohydrate content of infected plants which may reflect the alteration in the different metabolic processes favourable or unfavourable for fungal development. The decrease in the level of soluble and insoluble carbohydrates in leaves as compared with healthy ones was probably due to the increase in respiration of treated plants and/or increase in dehydrogenase and pentose cycle enzyme activities\(^{33}\). Depletion of starch in the chloroplast could be attributed to sporulation or likely to be due to the decrease in photosynthetic efficiency.} 

\textbf{Phenol content—}\textit{Treatment of leaves with A.sesami led to a highly significant increase \((P < 0.01\) or \(0.05\)) in total phenol content when compared to the healthy control plants (Table 3). However, no significant difference was observed in these contents between healthy and infected leaves of Thilarani at 5 and 10 days after inoculation. On the other hand, wild infected species had higher levels of phenolic compounds particularly after 10 days of inoculation. Generally, the concentration of total phenols increased as time progressed. Phenolic compounds might have unlimited potential in accounting for the} 

<table>
<thead>
<tr>
<th>Pigments</th>
<th>Wild Control</th>
<th>Infected</th>
<th>Thilarani Control</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Chl</td>
<td>46.8±0.02</td>
<td>25.1±0.44(^a)</td>
<td>31.04±0.17</td>
<td>16.1±0.01(^a)</td>
</tr>
<tr>
<td>Chl a</td>
<td>21.5±0.07</td>
<td>12.4±0.51(^a)</td>
<td>14.08±0.34</td>
<td>8.6±0.33(^a)</td>
</tr>
<tr>
<td>Chl b</td>
<td>28.6±0.13</td>
<td>14.8±0.09(^a)</td>
<td>15.8±0.08</td>
<td>9.9±0.47(^a)</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>5.4±0.43</td>
<td>8.9±0.26(^b)</td>
<td>3.7±0.11</td>
<td>5.4±0.69(^b)</td>
</tr>
</tbody>
</table>

Significant at \(^a\)0.05 or \(^b\)0.01 levels

<table>
<thead>
<tr>
<th>Wild</th>
<th>Thilarani</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing sugar ((\mu g/g) fresh weight)</td>
<td>0.68±0.01</td>
</tr>
<tr>
<td>Sucrose ((\mu g/g) fresh weight)</td>
<td>4.45±0.03</td>
</tr>
<tr>
<td>Polysaccharides ((\mu g/g) fresh weight)</td>
<td>9.83±0.1</td>
</tr>
<tr>
<td>Protein ((mg/g) tissue)</td>
<td>1.30±0.05</td>
</tr>
<tr>
<td>Proline ((\mu mol/g) fresh weight)</td>
<td>262.0±0.22</td>
</tr>
<tr>
<td>Free amino acids ((mg/g) fresh weight)</td>
<td>1.56±0.01</td>
</tr>
<tr>
<td>Disease related proteins ((mg/g) fresh weight)</td>
<td>0.08±0.001</td>
</tr>
</tbody>
</table>

Significant at \(^a\)0.05 or \(^b\)0.01 levels
many differences that occurred in plant response to disease. The defensive strategy of plants exists in two stages: the first is assumed to involve the rapid accumulation of phenols at the infection site, which function to slow the growth of the pathogen. The second would involve the activation of specific defenses such as the synthesis of phytoalexins or other stress-related substances. The present results confirm this conviction since a spectacular increase of phenols followed the treatment with the pathogens formulation as compared to the healthy leaves. Phenolic acid content revealed by RP-HPLC also showed variation between the infected and control sesame species. Lattanziol et al. reviewed that phenolics in host plants increase the tolerance limits. They reported the existence of p-coumaric, chlorogenic, vanillic, ferulic, protocatechuic, and p-hydroxybenzoic acids in healthy leaves. In fact, in infected plants, phenolics are substrates for the synthesis of compounds involved in disease resistance, like pterocarpan phytoalexins and hydroxycinnamic acid esters and for the production at or near the infection site of bioreistant phenylpropanoid polymers (lignins and suberins) which act to scar over the wound and as a barrier to the penetration or the propagation of the pathogen.

Protein, free amino acid and proline content—The protein concentration like that of carbohydrates was found to be decreased in infected cultivar species when compared to infected wild and controls (Table 2). The data was statistically significant at _P_ < 0.01 or _P_ < 0.05 levels. Amino acids are important nutrients that are transferred from the host plant to the pathogen and can function as nitrogen sources for the fungus. Meanwhile, the total amino acid level increased in the infected leaves. Low protein content and higher free amino acid content in diseased samples indicate that the fungal infection may cause denaturation or proteolysis of proteins and bound amino acids, resulting in an enhanced free amino acid content of the host tissues. High disease related protein level in fungus infected wild sesame leaf tissues, suggesting the active phase of protein synthesis in the host. Rampitsch et al. reported that the decrease or complete disappearance of certain amino acids may be either due to the utilization by the pathogen or have been utilized by the host plant for the defense mechanism.

A higher amount of proline was observed in the infected wild and cultivar sesame compared to the respective controls, suggesting its role as stress amino acid (Table 2). Proline is a major component of structural proteins or as osmoprotectant capable of mitigating the impacts of drought, salt, temperature and pathogenic stress in plants. When plants are exposed to pathogenicity, they produce reactive oxygen species (ROS) that induce programmed cell death in the plant cells surrounding the infection site and also effectively wall of the pathogen and terminate the disease process. On the other hand, proline may also act as a potent scavenger of ROS and this property might prevent the induction of programmed cell death by ROS. Similarly, proline may also function as a protein-compatible hydrotrope and as a hydroxyl radical scavenger. In any way, the higher proline accumulation in diseased tissue as

<table>
<thead>
<tr>
<th>Phenolic acid</th>
<th>Wild Control</th>
<th>Wild Infected</th>
<th>Thilarani Control</th>
<th>Thilarani Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinapic acid</td>
<td>1388.9±0.67</td>
<td>2205.9±0.78</td>
<td>1765.01±0.69</td>
<td>2039.89±0.72</td>
</tr>
<tr>
<td>Caumaric acid</td>
<td>1816.29±0.98</td>
<td>2877.0±0.45</td>
<td>2302.6±0.53</td>
<td>2661.0±0.48</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>188.89±0.66</td>
<td>50.91±0.22</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vanillate</td>
<td>188.89±0.59</td>
<td>203.53±0.25</td>
<td>240.4±0.69</td>
<td>245.07±0.55</td>
</tr>
<tr>
<td>Gallate</td>
<td>161.91±0.49</td>
<td>188.2±0.38</td>
<td>222.3±0.67</td>
<td>220.74±0.19</td>
</tr>
<tr>
<td>Chlorogenate</td>
<td>188.89±0.28</td>
<td>200.9±0.53</td>
<td>-</td>
<td>235.64±0.48</td>
</tr>
<tr>
<td>Ferulate</td>
<td>136.4±0.29</td>
<td>159.91±0.35</td>
<td>-</td>
<td>737.56±0.98</td>
</tr>
<tr>
<td>Phloroglucinol</td>
<td>179.8±0.26</td>
<td>824.5±0.12</td>
<td>-</td>
<td>967.08±0.87</td>
</tr>
<tr>
<td>Catechol</td>
<td>234.47±0.77</td>
<td>61.46±0.38</td>
<td>285.5±0.482</td>
<td>291.04±0.51</td>
</tr>
<tr>
<td>Hydroxyl benzoic acid (HBA)</td>
<td>34.13±0.26</td>
<td>85.15±0.17</td>
<td>43.9±0.35</td>
<td>164.67±0.36</td>
</tr>
<tr>
<td>Total phenol</td>
<td>22.04±0.09</td>
<td>44.24±0.07</td>
<td>16.98±0.05</td>
<td>29.58±0.02</td>
</tr>
</tbody>
</table>

Significant at _P_ < 0.05 or _P_ < 0.01 levels
noted in the present study might be related to its pathological resistance.

The SDS-PAGE protein profile of total soluble proteins from diseased leaves of both wild and Thilarani species showed differences in band patterns when compared with their respective healthy plants (Fig. 6). In wild species the fungal infection caused the disappearance of protein bands at 30, 49 and 150 kDa which were present in the healthy plant, while some new protein bands at 22, 25 and 110 kDa were observed in diseased samples that were absent in the healthy species. In cultivar species, 25 kDa protein band, pronounced in healthy plants, appeared to be absent in diseased material. Moreover, two protein bands of 27 and 28 kDa appeared to be hypersensitive in healthy plants as compared to diseased. Resistance-associated proteins are reported in several pathogen-host interactions. Plant pathogens such as viruses, bacteria, fungi and nematodes elicit the synthesis of host proteins which help in restricting the multiplication and spread of pathogens in the healthy tissue. Plants have flexible detection systems and probably employ several recognition and signal transduction pathways to activate their defense. Overall, precise temporal and spatial coordination of induced defense responses are required to successfully kill or restrict the invading microbe while simultaneously minimizing the damage to host tissue.

Isozyme pattern—The induction and change in the isozyme profile is considered to play an important role in the cellular defense against oxidative stress, caused by pathogen infection. Among these enzymes, CAT, EST, ACP, POX, PPO and SOD isozymes in both Thilarani and wild sesame subjected to A. sesami infection showed varied responses. When protein extracts separated by native electrophoresis, one CAT isoenzyme in varying density and position was observed in infected wild sesame plant (Fig. 7 a).

![Fig. 6 — SDS-PAGE profile of total proteins from control and infected leaves of *S. orientale*-wild and cultivar-Thilarani.](image)

![Fig. 7 (a-f) — Isozyme polymorphism profile from infected and control leaves of *S. orientale* wild and cultivar-Thilarani. a: Catalase (CAT), b: Peroxidase (POX), c: Esterase (EST), d: Polyphenol oxidase (PPO), e: Superoxide dismutase (SOD), f: Acid phosphatase (ACP).](image)
This result is in consistent with the report of Chatterjee and Ghosh. This band is dissimilar in its mobility from the respective control. POX isozyme in wild sesame infected leaves showed two new bands with higher density and position compared with three bands in the control. Infected Thilarani leaves displayed only a single major band with increased density. However, control showed two bands (Fig. 7 b). In the case of EST band profiling, a clear extra bands were found in both diseased plants (one in wild and two in Thilarani), and also the hyperactive bands observed in diseased plants suggest higher enzyme activity compared with their respective healthy plants (Fig. 7 c). For PPO and SOD the bands were found to be hyperactive in diseased plants in comparison with control plants, whereas in case of ACP the reverse was noticed (Fig. 7 d, e and f).

Antioxidant enzyme activity—Superoxide dismutase (SOD) converts the first product of the univalent reduction of \( \text{O}_2^- \) to \( \text{H}_2\text{O}_2 \), which must then be processed by CAT and/or peroxidases. Therefore, the significant increase in the levels of SOD during pathogenic infection suggests production of considerable amounts of superoxide and a proportional response by the stressed sesame (Table 4). Increased levels of SOD are reported from *Nicotiana benthamiana* to infection with two strains of pepper mild mottle virus. The data was statistically significant at \( P<0.01 \) or \( P<0.05 \) levels. CAT activity in wild sesame provides circumstantial evidence to support the hypothesis that infection causes the formation of ROS. In Thilarani, the activity of CAT was increased significantly compared with control (Table 4). The present data proved that *A. sesami* induces specific responses from the plant antioxidant defense system including the varied activity of CAT. The present results are in agreement with catalase activities in different sugar beet genotypes infected with root-knot nematode and bacterial spot pathogenesis in tomato.

The induction of peroxidase (POX) activity has been repeatedly reported in several plant species in response to pathogen infection. POX activity, in general increases under different stress conditions like wounds, fungal infections, salinity, water stress and nutritional disorders, inducing also the lignin increment and production of ethylene and induce the increase of the production of phenols oxidized at the cell wall. This activity, suggests a cell effort for the establishment of a physiochemical barrier, able to isolate the infected area. In the present study, the induction of POX activity in infected sesame leaf of wild and Thilarani over their controls has been revealed (Table 4). The data was statistically significant at \( P<0.01 \) or \( P<0.05 \) levels. The highest induction was recorded over control in wild sesame. However, not much substantial increase in enzyme activity was observed in the Thilarani throughout the infection. The enhancement of POX in sesame leaf upon *A. sesami* infection herein was in agreement with that reported for taro upon infection with *Phytophthora colocasiae* and *Cucumis sativus* upon inoculation with cucumber downy mildew *P. cubensis* where a positive correlation between the enhancement of POX activity and the degree of plant resistance towards the pathogen was recorded.

Esterases (EST) are usually used to measure genetic variation, yet they may also be influenced by external factors. Elevated differential EST enzyme activity in both the diseased plants compared to their respective controls against *A. sesami* suggests that the biochemical traits are genetically based (Table 4).

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Wild (U/mg protein)</th>
<th>Infected (U/mg protein)</th>
<th>Control (U/mg protein)</th>
<th>Infected (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT</td>
<td>20.1±0.38</td>
<td>42.2±0.46</td>
<td>8.3±0.05</td>
<td>19.0±0.03</td>
</tr>
<tr>
<td>POX</td>
<td>19.4±0.27</td>
<td>29.8±0.04</td>
<td>5.1±0.06</td>
<td>8.6±0.008</td>
</tr>
<tr>
<td>SOD</td>
<td>6.15±0.11</td>
<td>18.5±0.01</td>
<td>1.45±0.01</td>
<td>2.0±0.004</td>
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<tr>
<td>ACP</td>
<td>1.1±0.001</td>
<td>0.072±0.02</td>
<td>0.7±0.001</td>
<td>0.56±0.002</td>
</tr>
<tr>
<td>EST</td>
<td>0.06±0.003</td>
<td>0.09±0.001</td>
<td>0.035±0.002</td>
<td>0.06±0.001</td>
</tr>
<tr>
<td>PPO</td>
<td>542.4±0.82</td>
<td>448.8±0.59</td>
<td>178.8±0.26</td>
<td>343.8±0.44</td>
</tr>
</tbody>
</table>

Table 4 — Activity of antioxidant enzymes—catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), acid phosphatase (ACP), esterase (EST) and polyphenol oxidase (PPO) in infected and control leaves of sesame—wild & Thilarani species.

Values are with ± SD from 6 experiments each.

Significant at \( ^a0.05 \) or \( ^b0.01 \) levels.
Polyphenol oxidase (PPO) activity displayed a decrease in *A. sesami* infected wild plants. The assay data corroborates with the isozyme pattern and phenol content in the plants. The elevated PPO profile convert phenols into quinone derivatives which will indirectly affecting the fungal penetration in the host tissue (Table 4).

Acid phosphatase (ACP) expression is induced due to factors like water deficit, salt, metal or pathogen stresses. The induction could be secondary to inorganic phosphate generating enzymes, like PFK and PEP carboxylase. Sesame infected with *A. sesami* showed decreased ACP activities. However, the decrease was more pronounced in Thilarani than in wild species. Sharma and Kaur\(^{50}\) observed that acid phosphatase are known to act under salt and water stress by maintaining a certain level of inorganic phosphate which can be co-transported with H\(^+\) along a gradient of proton motive force. In few cases, phosphatase activities are independent of phosphate levels. The depleted level of ACP in sesame is suggestive of a poor sensor mechanism for pathogen stress endurance. Probably, pathogenicity might have inhibited the signal for elevated expression of ACP.

Since enzymes control biochemical reactions, and their synthesis are under the control of specific gene(s), any change in the activity of an enzyme would reflect the pattern of gene expression and corresponding metabolic events in the cell. Hence, enzymes can be used as tools to study the induced responses of plants showing disease symptoms at the biochemical level\(^ {51}\). In addition, phenol-oxidizing enzymes such as POX and PPO are correlated with many diseases\(^ {43}\). In the present investigation, changes in the activities of CAT, EST, ACP, POX, SOD and PPO along with total amount of protein have been studied in sesame to understand the fate of existing biochemical components in these plants upon infection by *A. sesami*. ACP catalyzes the hydrolysis of phosphate esters with consequent release of inorganic phosphate and plays an important role in phosphorus metabolism\(^ {44}\). Thus, the present study reveals that this normal metabolism of ACP was found to be hampered due to infection. Significant level of EST and SOD activity in diseased wild species of sesame indicates a probable mechanism of overcoming the oxidative stress situation developed due to fungal infection. Altered zymogram patterns of catalases suggest inactivation of existing catalases, activation of an inactive form and/or synthesis of new catalases. The appearance of new isozymes of CAT in infected tissue might play a unique role in disease resistance. The higher activity of POX, a marker enzyme of lignin synthesis in diseased plants probably resulted in up - regulating the metabolic pathway for ligno-cellulosic cell wall formation, thereby providing a possible resistance for the fungal infection into host cell wall. Based on the differential ability of PPO and POX to drive the oxidation and condensation of lignin precursors, it has been suggested that PPO might be primarily responsible for the initial polymerization of monolignols into oligolignols\(^ {45}\) whereas POX would be more likely to catalyze the reactions leading from oligolignols to highly condensed macromolecular lignin.

APO activity is ubiquitous in higher plants, and functions attributed to the enzyme include phenol metabolism and a defense mechanism against pathogens\(^ {44}\). The hyperactive profiling of PPO in diseased plants is normally associated with an improved host defense mechanism\(^ {43}\) but in the present investigation the host defense system in sesame cultivars appeared to have totally failed despite the enhanced PPO activity observed in diseased leaves compared to the wild. The reason behind such a situation is still unknown and warrants further study.

In conclusion, this study provides information related with the hyphal structure, penetration and colonization of *A. sesami* in *S. orientale*. Similarly, the observed biochemical alterations associated with the infection suggest that, in the first stage, the plant uses unspecific mechanisms to eliminate the fungus, such as an increase in free amino acids, carotenoids and phenolics. However, these mechanisms seem not to be sufficient to avoid the disease and a number of other biochemical changes, such as varied activities of antioxidative enzymes, its isozyme pattern and pathogen related proteins suggest that a cascade of events has been triggered by the pathogen in *S. orientale* to develop a hypersensitive response in the infected regions of the plant. To verify these findings and advance understanding of this complex pathogen–host interaction as well as the detailed analysis of gene expression of both pathogenicity factors and host response factors will be required. Along these lines, gene expression is currently being analyzed in cDNA libraries generated from resistant
and susceptible cultivars after inoculations with A. sesami is an attempt to understand the gene expression during pathogenesis.

References


