Defensive Responses of Rice genotypes for Resistance Against Rice Leaffolder *Cnaphalocrocis medinalis*

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**Abstract:** The experiment was carried out to assess the reaction of different categories of rice genotypes viz., resistant, susceptible, hybrid, scented, popular and wild in response to the infestation by rice leaffolder (RLF), *Cnaphalocrocis medinalis* (Guenee) and to explore the possible use of these genotypes in developing RLF-resistant rice varieties. The changes of various biochemical constituents such as soluble leaf protein, phenol, ortho-dihydroxy phenol, tannin and enzymes viz., peroxidase, phenyl alanine ammonia lyase (PAL) were assessed spectrophotometrically in all the rice genotypes before and after RLF infestation. The protein profile was analyzed using sodium dodecyl sulphate-poly acrylamide gel electrophoresis (SDS-PAGE) method. A significant constituent of biochemical content such as tannin, phenol, ortho-dihydroxy phenol has been increased along with enzyme activities of peroxidase and PAL in the infested resistant (Ptb 33, TKM6 and LFR831311) and wild rice genotypes (*Oryza minuta* and *O. rhizomatis*). A decrease in leaf protein content was evident invariably in all the infested rice genotypes. It is also evident that the content of biochemicals such as phenol, ortho-dihydroxy phenol and tannin were negatively correlated with leaffolder damage. However, leaf protein content was positively correlated with the damage by rice leaffolder. SDS-PAGE analysis for total protein profiling of healthy and *C. medinalis*-infested genotypes revealed the enhanced expression of a high molecular weight (> 97 kDa) protein in all the genotypes. Besides, there was also an increased induction of a 38 kDa protein in *C. medinalis* infested resistant genotypes, which was absent in uninfested plants. The present investigation proved that the elevated levels of biochemicals and enzymes may play a vital role in rice plants resistance to RLF.

**Key words:** rice genotype; biochemical; defense enzyme; rice leaffolder; resistance

Rice is one of the most important staple food crops and is grown extensively in different parts of the world. The rice leaffolder (RLF), *Cnaphalocrocis medinalis* (Guenee) was considered as minor or sporadic pest in the past in many Asian countries. But, now it has assumed as one of important insect pests and became a major threat to rice production in tropical and subtropical Asia. Reports show that severe infestation of this pest leads to the leaf damage as high as 60 to 70 per cent (Kushwaha and Singh, 1984), causing significant yield losses (Shrivastava, 1989).

Insect and the pathogen attack are the major constrains that a plant faces. Majority of plants employ different defense tactics and these can influence herbivore settling, feeding, oviposition, growth, development, fecundity and fertility (Baldwin, 1999). Accumulation of defense enzymes, chemicals, resistant proteins by insect feeding has been reported in many insect-plant interactions (Radja Commare et al, 2002). The plant strategy to deter feeding herbivore has become an important aspect of insect-plant interaction studies and is gaining tremendous importance.

Feeding activities of herbivorous insects often result in physiological, morphological and chemical changes in the form of accumulation of the compounds having defensive properties. The biochemical factors are chemicals that affect insect behaviour, physiology and growth. Some biochemical factors are associated with repellence, feeding deterrence toxicity or adverse effects on insects (Saxena, 1986). Among the plant chemicals, presence of increased or decreased amount of both the nutritional compounds and non nutritional secondary substances influences the resistance or susceptibility of plants to insects (Bharathi, 1996). According to Kogan and Paxton (1983), many changes
that occur following herbivory result in accumulation of phenolic compounds. Also, the direct defense is known to reduce insect growth rates by interfering with the digestibility and nutritive quality of plant tissues (Johnson et al., 1989). An understanding of the underlying chemical mechanisms might have contributed to the development of varieties with a more durable resistance.

Identification of biochemical markers associated with the resistance to RLF would help in enhancing rice breeding efficiency. Levels of peroxidase expression and its isoenzyme patterns have been shown in several plant systems like rice, tobacco, wheat and barley due to stress, chemicals and infection (Gasper et al., 1982). In this context, the accumulation of defense molecules in resistant and susceptible genotypes has been exploited to utilize the defense mechanisms in integrated pest management in rice. The induced response in plant biochemistry and molecular biology of signal transduction, and phytochemical responses has been studied in only a very limited number of plants. Biochemical studies of rice varieties will be helpful in confirming the physiological antibiosis of the new rice germplasm. Hence, the present study was undertaken to study the activities of defense molecules in resistant and susceptible genotypes and their effects on RLF infestation.

MATERIALS AND METHODS

Rice materials and culture of *C. medinalis*

Seeds of susceptible rice varieties (TN1, IR36) (Khan et al., 1989), moderately resistant variety (ASD16), resistant varieties (TKM6, Ptb 33, LFR831311) (Nadarajan and Nair, 1983; Rajendran et al., 1986) (2001), scented variety (Pusa Basmati), hybrid (CORH1), popular variety (ADT36) and wild rice genotypes (*Oryza minuta* and *O. rhizomatis*) were collected from Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore, India. Seeds were sown in mud pots (10 cm × 12 cm). Rooted slips of the wild rice genotypes were planted in tubular mud pots (80 cm × 80 cm) and grown in insect proof green house. *C. medinalis* was cultured according to Waldbauer and Marciano (1979). The adults collected in fields were released to the oviposition cages (50 cm × 50 cm × 75 cm) inside which 30-day-old potted plants of TN1 and honey solution (20%) as adult feed were kept in plastic trough containing water. The eggs were collected daily and placed on moist filter paper in Petri dishes. Then the newly hatched first instar larvae were transferred singly to the axils of rice plants with a fine pointed camel hair brush. The larvae fed on the leaves by scraping the green matter. The suitable size larvae were transferred in each rice genotype for biochemical analysis.

Estimation of biochemicals and enzymes

All rice genotypes were analyzed for their biochemical constituents at the peak vegetative stage by collecting 1 g of sample per genotype from the second and the third leaves. Biochemical analysis was also made to determine the variation in the biochemical constituents of rice genotypes before and after the RLF infestation, by two 3rd instar larvae on the top leaves. After 15 d, the leaf samples were collected from the 2nd and the 3rd leaves of healthy and infested plants and stored at −70 °C for the estimation of biochemical components. The phenol, ortho-dihydroxy phenol, tannin and protein contents were estimated using the methods of Malick and Singh (1980), Johnson and Scheal (1957), Bwins (1971) and Lowry et al. (1951), respectively. For determining enzyme activities viz., peroxidase and phenyl alanine ammonia lyase (PAL), 1 g of leaf samples was homogenized with suitable buffer solution as per the method described by Sadasivam and Manickam (1996).

Analysis of protein profile

Analysis of the protein profile was carried out by 12% sodium dodecyl sulphate-poly acrylamide gel electrophoresis (SDS-PAGE) method according to Lagrimini and Rothstein (1987). Protein samples were prepared by grinding 1.0 g leaf samples with 0.1 mol/L phosphate buffer in a pre-chilled mortar and pestle. Protein samples equivalent to 200 µg each were mixed with equal volume of sample buffer and heated to 40 ºC for 1 min. After cooling to room temperature, the samples were centrifuged at 1 000 r/min for 2 min. The supernatant was loaded on the gel and electrophoresis was carried out at 20 ºC. The gel was stained for 4 h. It was then destained till the background was colourless and the bands became clearly visible. The destained gel was preserved in 7% acetic acid solution and then photographed.

Statistical analysis

The data obtained from various experiments were statistically analyzed in a completely randomized block design and different parameters observed in the experiments were subjected to Duncan’s Multiple Range Test (DMRT) \( (P = 0.05) \) analysis using IRRISTAT.
version 92-a, developed by International Rice Research Institute Biometrics Unit, the Philippines.

RESULTS

Phenol and ortho-dihydroxy phenol content

The total phenol content in healthy and RLF-infested leaves was found to range from 3.48 to 10.83 mg/g and 4.31 to 14.08 mg/g, respectively (Table 1). Resistant genotypes Ptb 33 and O. minuta registered the maximum of 10.83 and 9.86 mg/g total phenol in healthy, and 14.08 and 12.94 mg/g total phenol in the infested plants, respectively. However, susceptible genotypes had only 3.48 and 3.54 mg/g total phenol in healthy, and 4.31 and 4.46 mg/g total phenol in infected plants in TN1 and IR36, respectively. The infestation by RLF resulted in an increase in total phenol content at 44.09% and 38.14% in O. rhizomatis and LFR831311, respectively, in compared with 23.85% and 24.25% in TN1 and Pusa Basmati.

Tannin and protein content

Tannin content ranged from 0.93 to 1.89 mg/g in healthy and 1.19 to 2.89 mg/g in the infested rice genotypes.

Table 1. Phenol and ortho-dihydroxy phenol content of healthy and C. medinalis infested plants of selected rice genotypes.

<table>
<thead>
<tr>
<th>Rice genotype</th>
<th>Healthy plant (mg/g)*</th>
<th>Infested plant (mg/g)*</th>
<th>Increase due to leaffolder damage (mg/g)</th>
<th>Increase (%)</th>
<th>Healthy plant (mg/g)*</th>
<th>Infested plant (mg/g)*</th>
<th>Increase due to leaffolder damage (mg/g)</th>
<th>Increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TKM6</td>
<td>9.56</td>
<td>12.46</td>
<td>2.90</td>
<td>30.33 d</td>
<td>2.62</td>
<td>3.87</td>
<td>1.25</td>
<td>47.70 a</td>
</tr>
<tr>
<td>Ptb 33</td>
<td>10.83</td>
<td>14.08</td>
<td>3.25</td>
<td>30.00 d</td>
<td>2.58</td>
<td>3.65</td>
<td>1.07</td>
<td>41.47 d</td>
</tr>
<tr>
<td>CORH1</td>
<td>6.82</td>
<td>7.96</td>
<td>1.14</td>
<td>16.71 i</td>
<td>1.92</td>
<td>2.43</td>
<td>0.51</td>
<td>26.56 f</td>
</tr>
<tr>
<td>Pusa Basmati</td>
<td>7.09</td>
<td>8.81</td>
<td>1.72</td>
<td>24.25 g</td>
<td>1.88</td>
<td>2.29</td>
<td>0.41</td>
<td>21.80 h</td>
</tr>
<tr>
<td>LFR831311</td>
<td>9.36</td>
<td>12.93</td>
<td>3.57</td>
<td>38.14 b</td>
<td>2.65</td>
<td>3.87</td>
<td>1.22</td>
<td>46.03 b</td>
</tr>
<tr>
<td>ASD16</td>
<td>6.93</td>
<td>8.85</td>
<td>1.92</td>
<td>27.70 e</td>
<td>1.99</td>
<td>2.49</td>
<td>0.50</td>
<td>25.12 g</td>
</tr>
<tr>
<td>TN1</td>
<td>3.48</td>
<td>4.31</td>
<td>0.83</td>
<td>23.85 h</td>
<td>1.89</td>
<td>2.29</td>
<td>0.40</td>
<td>21.16 i</td>
</tr>
<tr>
<td>IR36</td>
<td>3.54</td>
<td>4.46</td>
<td>0.92</td>
<td>25.98 f</td>
<td>1.96</td>
<td>2.34</td>
<td>0.38</td>
<td>19.38 j</td>
</tr>
<tr>
<td>O. minuta</td>
<td>9.86</td>
<td>12.94</td>
<td>3.08</td>
<td>31.23 c</td>
<td>2.56</td>
<td>3.72</td>
<td>1.16</td>
<td>45.31 c</td>
</tr>
<tr>
<td>O. rhizomatis</td>
<td>8.21</td>
<td>11.83</td>
<td>3.62</td>
<td>44.09 a</td>
<td>2.68</td>
<td>3.66</td>
<td>0.98</td>
<td>36.56 e</td>
</tr>
<tr>
<td>Sed</td>
<td>0.12</td>
<td>0.19</td>
<td>0.08</td>
<td>2.46</td>
<td>NS</td>
<td>NS</td>
<td>0.06</td>
<td>2.25</td>
</tr>
</tbody>
</table>

* Means of three replications. Values were arc sine transformed and original values are given.

In a column mean followed by the same letter are not significantly different at $P = 0.05$ as per Duncan’s Multiple Range Test.

Table 2. Tannin and total protein content of healthy and C. medinalis infested plants of selected rice genotypes.

<table>
<thead>
<tr>
<th>Rice genotype</th>
<th>Healthy plant (mg/g)*</th>
<th>Infested plant (mg/g)*</th>
<th>Increase due to leaffolder damage (mg/g)</th>
<th>Increase (%)</th>
<th>Healthy plant (mg/g)*</th>
<th>Infested plant (mg/g)*</th>
<th>Decrease due to leaffolder damage (mg/g)</th>
<th>Decrease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TKM6</td>
<td>1.86</td>
<td>2.89</td>
<td>1.03</td>
<td>55.37 b</td>
<td>6.30</td>
<td>6.21</td>
<td>0.08</td>
<td>1.33 i</td>
</tr>
<tr>
<td>Ptb 33</td>
<td>1.73</td>
<td>2.68</td>
<td>0.95</td>
<td>54.91 c</td>
<td>6.49</td>
<td>6.38</td>
<td>0.12</td>
<td>1.80 f</td>
</tr>
<tr>
<td>CORH1</td>
<td>1.03</td>
<td>1.23</td>
<td>0.20</td>
<td>19.41 i</td>
<td>6.97</td>
<td>6.58</td>
<td>0.39</td>
<td>5.59 c</td>
</tr>
<tr>
<td>Pusa Basmati</td>
<td>1.06</td>
<td>1.41</td>
<td>0.38</td>
<td>35.84 f</td>
<td>7.13</td>
<td>6.83</td>
<td>0.30</td>
<td>4.21 d</td>
</tr>
<tr>
<td>LFR831311</td>
<td>1.89</td>
<td>2.92</td>
<td>1.03</td>
<td>54.49 d</td>
<td>6.52</td>
<td>6.41</td>
<td>0.11</td>
<td>1.70 h</td>
</tr>
<tr>
<td>ASD16</td>
<td>1.21</td>
<td>1.43</td>
<td>0.22</td>
<td>18.18 j</td>
<td>6.23</td>
<td>6.11</td>
<td>0.12</td>
<td>1.97 c</td>
</tr>
<tr>
<td>TN1</td>
<td>0.93</td>
<td>1.19</td>
<td>0.26</td>
<td>27.95 g</td>
<td>8.08</td>
<td>7.61</td>
<td>0.47</td>
<td>5.77 a</td>
</tr>
<tr>
<td>IR36</td>
<td>0.96</td>
<td>1.21</td>
<td>0.25</td>
<td>26.04 h</td>
<td>8.05</td>
<td>7.60</td>
<td>0.46</td>
<td>5.67 b</td>
</tr>
<tr>
<td>O. minuta</td>
<td>1.71</td>
<td>2.74</td>
<td>1.03</td>
<td>60.23 a</td>
<td>6.73</td>
<td>6.61</td>
<td>0.12</td>
<td>1.76 g</td>
</tr>
<tr>
<td>O. rhizomatis</td>
<td>1.86</td>
<td>2.61</td>
<td>0.75</td>
<td>40.32 e</td>
<td>6.42</td>
<td>6.31</td>
<td>0.11</td>
<td>1.69 b</td>
</tr>
<tr>
<td>Sed</td>
<td>NS</td>
<td>NS</td>
<td>0.05</td>
<td>2.93</td>
<td>NS</td>
<td>NS</td>
<td>0.03</td>
<td>0.24</td>
</tr>
</tbody>
</table>

* Means of three replications. Values were arc sine transformed and original values are given.

In a column mean followed by the same letter are not significantly different at $P = 0.05$ as per Duncan’s Multiple Range Test.
There was no significant difference observed in tannin content in the healthy and infested plants of selected genotypes. The resultant increase of tannin content due to RLF damage registered as high as 60.23% and 55.37% in *O. minuta* and TKM6, respectively, as against 18.18% and 19.41% in ASD16 and CORH1, respectively.

Soluble leaf protein content was estimated from the leaves of the healthy and infested plants ranged from 6.23 to 8.08 and 6.11 to 7.61 mg/g, respectively. In all rice genotypes, there was a decrease in soluble leaf protein content due to RLF infestation. It was high in TN1 (5.77 mg/g), while very low (1.33 mg/g) in TKM6. Though there was no significant difference in respect of change in protein content in rice genotypes, significant decrease in protein content could be seen before and after RLF infestation.

**Peroxidase and PAL activities**

The activities of peroxidase and PAL were found to increase in all the rice genotypes infested by RLF in comparison with the healthy genotypes (Figs. 1 and 2). But there was no significant difference observed between the resistant and susceptible rice genotypes. The change in peroxidase and PAL activities due to RLF infestation was calculated by subtracting the enzyme activity recorded in the infested plants from those healthy plants. Among the rice genotypes, maximum increase in both of the enzyme activities was observed in the susceptible genotypes of TN1 and IR36 as compared to resistant wild genotypes *O. minuta* and *O. rhizomatis*, respectively.

**Protein profile by SDS-PAGE method**

Total soluble protein profiling of the healthy and infested leaves of selected genotypes was analyzed through SDS-PAGE (Fig. 3). The results revealed an enhanced expression of a high molecular mass (> 97 kDa) protein in all infested varieties due to insect infestation. Besides there was an increased induction of a 38 kDa...
protein in infested resistant and moderately resistant genotypes (Ptb 33, TKM6, LFR831311, ASD16, *O. minuta* and *O. rhizomatis*), which was absent in their healthy plants profile. However, no such difference in protein level was noticed between the infested and healthy plants of susceptible varieties CORH I, ADT36 and Pusa Basmati.

**DISCUSSION**

Plant secondary metabolites act as chemical signals in the ecosystem and as antibiosis agents against insects and pathogens. Important secondary metabolites involved in insect resistance in higher plants are phenolic compounds like phenylpropanoids. So analysis of biochemical compositions in susceptible and resistant genotypes in the present study helped us to understand why the genotypes were differentially damaged and the cause of resistance or otherwise.

The present study explored the insect-feeding induced damage on rice plants and its subsequent effects on the plant biochemical and enzymatic changes in the form of quantitative changes. In most of the infested resistant rice genotypes *viz.*, Ptb33, TKM6, LFR831311, *O. rhizomatis* and *O. minuta*, the levels of biochemical such as phenol, ortho-dihydroxy phenol and tannin contents increased obversely compared with ones in the susceptible genotypes of TN1 and IR36. On contrary, there was a slight decrease in the protein content invariably in all the infested genotypes compared with the healthy genotypes. The high total phenol, ortho-dihydroxy phenol and tannin contents are responsible for substantial increase in defensive mechanism on the basis of antibiosis, which consistently inhibiting the oviposition, population build up, survival and adult emergence of rice leaffolder. This was proved by correlation analysis of biochemicals such as phenol, ortho-dihydroxy phenol and tannin contents were negatively correlated with RLF damage, while the leaf protein content was positively correlated (Fig. 4). This is in agreement with the earlier findings that high phenol content in rice is negatively correlated with the incidence of the whitebacked planthopper (Rath and Misra, 1998), thrips (Thayumanavan et al., 1990), leaffolder (Mohan et al., 1988), brown planthopper (Grayer et al., 1994), rice gall midge and stem borer (Vidyachandra et al., 1981).

An increase in phenolic compounds is considered to be a common reaction to insect attack. The increased phenol content is correlated with its negative effects on the larva as explained by Haukioja and Niemela (1977). Feeding by insects changed the phenol content in the rice plants. Lygus bug feeding on sugar beets resulted in increased quinines, which inhibited subsequent bug feeding (Hori, 1973). There were similar results as there was rise in the concentration of phenolic acids like vanillic acid, syringic acid, cinnamic acid, and p-coumaric acid being observed in
infested rice plants. Usha Rani and Jyothsna (2009), Jyothsna et al (2009) and Felton et al (1992) found that the increased concentration in phenolic compounds is according to the extent of tissue damaged by feeding insects.

In addition, the present study demonstrated that the protein content in the resistant genotypes was lower as compared to ones in the susceptible genotypes. However, the difference in protein content was not significant between the susceptible and resistant genotypes studied. But significant reduction in soluble leaf proteins was noticed in the infested lines compared to their healthy counter parts. The protein content has been reported to indirectly influence resistance in rice plants. Similar results have also been reported by Beck et al (1983) on corn leaves of resistant and susceptible varieties. Raghumooorthy and Gunathilagaraj (1988) reported that the amount of total seed proteins was less in the resistant rice varieties CO1, CO24 and CO32 to angoumois grain moth *Sitotroga cereallela*. Edwards and Wratten (1983) also observed decrease in protein content due to insect attack. Hori and Atalay (1980) observed that there was increase in protein up to the 3rd day after infestation in cabbage by *Lygus disporsi* bug, but from the 7th day, the protein content started decreasing. The initial increase in protein may be due to over expression of defense related protein. However, the decrease of leaf protein below control level could not be explained.

Though there was an overall decline in the soluble leaf protein content due to leaffolder infestation, a protein of high molecular weight (> 97 kDa) increased in all the selected genotypes after leaffolder infestation (Fig. 3). An interesting result of this study was that one more protein of 38 kDa was observed, which may be a defense related protein. From these results, it may be concluded that: there was an increase in expression of a specific protein due to leaffolder infestation, which may act as a key in identification of leaffolder tolerant or resistant genotypes. Increase in expression of defense related protein after infestation has been reported earlier and the results are in conformity with findings of Seema Sinha et al (2005).

In the present study, greater activities of enzymes such as peroxidase and PAL were recorded in resistant genotypes than susceptible genotypes after RLF infestation. Though there were increased activities of peroxidase and PAL due to RLF infestation, no significant difference was found between susceptible and resistant genotypes. These findings are in line with the findings of Seema Sinha et al (2005). Peroxidase plays an important role in the regulation of plant cell elongation, phenol oxidation, polysaccharide cross-linking, cross linking of extension monomers, oxidation of hydroxyl-cinnamyl alcohols into free radical intermediates and wound healing. The early and increased expression of peroxidase involved in the biochemical reaction necessary for lignification has protected plant from *C. medinalis* in rice. Hori and Atalay (1980) reported seven times increase in peroxidase activity that continued up to 21 d after injury. The increase in peroxidase activity in plants may be associated with lignification of wound by polymerization of p-coumaryl and coniferyl alcohol.

PAL catalyses the biosynthesis of phenolics, coumarins, isoflavonoids and lignin, which have an important role in insect resistance mechanism. Increase in PAL activity due to pathogen attack and wounding was observed by Jones (1984). Cahill and Mc-Comb (1992) have reported that increase in PAL activity due to *Phytophthora cinnamona* infection was more in resistant varieties compared to susceptible ones. Southerton (1990) also observed direct correlation of induction of PAL activity with phenol content. Our results confirmed that the damage by RLF to rice genotypes triggered changes in foliage quality in the rice genotypes, which might affect subsequently on the herbivore fitness of the attacked genotypes.
CONCLUSIONS

Plants respond to physical and chemical changes associated with insect feeding, through the accumulation of phenolic compounds and in accordance with the kind and degree of damage, diverse phenols are induced which involve enzymes such as PAL and peroxidases. Increasing instances are evident in several insect-plant systems wherein induced defenses play a useful role for development of insect resistant varieties. These defensive biochemical compounds and proteins might have reduced the pest incidence and simultaneously increase the yield in rice. In this context, the accumulation of defense molecules in resistant and susceptible genotypes has been exploited to utilize the defense mechanisms in integrated pest management in rice. In future, chemical profiling of rice varieties will be helpful in confirming the physiological antibiosis of the new germplasm of rice.

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