Studies on structural and biochemical mechanism of resistance in groundnut to *Puccinia arachidis*

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ABSTRACT: Mechanism of resistance on the basis of structural and biochemical changes in resistant (GPBD-4 and DH-22), moderately resistant (K-134 and R-8808) and susceptible (KRG-1 and TMV-2) genotypes of groundnut were studied. Resistant and moderately resistant genotypes were characterized by higher cuticular and epidermal cell thickness with lesser epidermal cells, size (length, breadth) and number of stomata and more wax content at later stages of crop growth. Resistant and moderately resistant genotypes recorded more sugars, phenol, ortho-dihydroxy phenol and protein contents than susceptible ones.

Key words: Groundnut, rust, *Puccinia arachidis*, resistance

Rust of groundnut (*Puccinia arachidis* Speg.) is an important disease of groundnut in India. The disease can cause yield losses of over 52 per cent and its incidence ranges from 19 to 50 per cent in all major groundnut growing areas of India (Subramanyam et al., 1980; Mayee, 1983). Many fungicides have been reported to control the disease (Vidyasekharan, 1981; Adiver et al., 1995; Jadeja et al., 1999). However, exploitation of host resistance would be an ideal approach in the context of subsistence farming of resource-limited semi-arid tropical regions of the world. Before formulation of a suitable disease resistant breeding strategy, understanding the basic mechanisms associated with resistance becomes necessary.

A great deal of work has been done on the expression and mechanisms of resistance at the site of infection or upon challenging virulent pathogen on the host in many leguminous crops (Deverall and Dann, 1995). Host resistance plays a vital role in management of rust as several sources of resistance in cultivated *Arachis* spp. have been identified (Subrahmanyam and McDonald, 1983; Mayee and Datar, 1988). However, the structural and biochemical mechanism of resistance to rust in groundnut has not been clearly understood. Nevill (1980) reported that *P. arachidis* is able to penetrate even the immune wild species, although development ceases after formation of a single hypha. In contrast, Cook (1980) found that the germ tube tended to pass over stomata without successful contact in resistant foliage. Many workers also noticed that resistance to foliage is characterized by specific morphological and anatomical features (Barsa et al., 1985 and Kaur and Dhillon, 1988). Further, it is reported that biochemicals and their oxidation products are implicated in disease resistance. The anti-microbial properties of biochemicals are due to rapid accumulation of their oxidized products in vital loci within the cell wall, which in turn brings about interference in the metabolism of the host as well as pathogen (Kuc, 1964). However, studies pertaining to groundnut rust are rather limited. The present study was therefore, undertaken to find out the role of structural and biochemical mechanisms involved in resistance against rust of groundnut.

MATERIALS AND METHODS

Six groundnut genotypes viz., GPBD-4 and DH-22 (resistant), K-134 and R-8808 (moderately resistant) and KRG-1 and TMV-2 (susceptible) of
groundnut were selected to study the histological and biochemical mechanisms of resistance in groundnut against rust disease. Each genotype was sown in 50 X 40 cm. cement pots with three replications. Four plants were maintained per pot. The fresh uredospore inoculum collected from the highly susceptible cultivar KRG-1 was inoculated at 40 DAS by stapler method. The plants were covered with transparent polythene bags for 24 h. The variation in temperature in the glass house ranged from 20-28°C. Middle leaves of genotypes were detached and taken for different histological studies.

**Structural mechanisms**

Structural mechanisms viz., cuticular thickness, epidermal cell thickness, number of epidermal cells, number and size (length, breadth) of stomata in different genotypes were studied following Varadarajan and Wilson (1973). A thin film of quickfix (adhesive) was smeared over the epidermis and spread slowly with the help of needle. After five minutes dried quick fix was slowly peeled off and mounted on clean slide and observed under microscope. Histological parameters viz., cuticular and epidermal cell thickness, number of epidermal cells, length, breadth and number of stomata of leaves of all the genotypes were recorded with the help of filar micrometer at 10X.

**Epicuticular Wax**

The wax content was determined by the method of Ebercon et al. (1977) which is based on the colour change produced by the reaction of wax with acidic potassium dichromate. The sample consisting of 20 groundnut leaf discs of known area (both surfaces) collected from 25, 50 and 75 days old plants were immersed in 15 ml. of chloroform for 15 seconds. The extract was filtered and evaporated to dryness on a hot water bath until a odour of chloroform could not be detected. Five ml of acidic K₂Cr₂O₇ was added to the sample and placed on hot water bath for 30 min. After cooling, 12 ml. of deionised water was added and allowed for 15 to 20 min. for colour development. The optical density of the sample was read at 590 nm. The wax content was quantified by using the standard curve prepared from Carbo wax 3000 and expressed as mg per dm² area. (inclusive of both surfaces since the wax is present on both the surfaces).

**Biochemical mechanisms**

The leaf material was extracted in alcohol as per the procedure given by Jaypal and Mahadevan (1968), for various biochemical analysis.

The biochemicals viz., sugars, phenol, ortho-dihydroxy phenol and protein present in healthy and diseased leaves of different genotypes were estimated as per standard procedures. The reducing sugar was estimated following Nelson’s modification of Somogyi’s method (Nelson, 1944). Non reducing sugars were hydrolysed using 1 ml 1N H₂SO₄ and then estimated as in the case of reducing sugars to get the total sugars. Non reducing sugars were calculated by subtracting the reducing sugars from that of total sugars. The estimation of total phenols and ortho-dihydroxy phenol present in plant samples was done by following Folin-Ciocalteau Reagent method and Arnow’s Reagent method respectively. Estimation of protein content in samples was done as per the procedure given by Lowry et al. (1951). Bovine serum albumin was used as the standard.

**RESULTS AND DISCUSSION**

**Structural mechanisms**

**Cuticular and Epidermal thickness and number**

The results revealed that, resistant and moderately resistant genotypes were characterized by significantly higher cuticular and epidermal cell thickness with lesser epidermal cells, length, breadth and number of stomata. The highest cuticular thickness (4.31 µm) was observed in GPBD-4 followed by Dh-22 (Red) (4.14 µm) and K-134 (3.56 µm). Further, GPBD-4 had more epidermal cell thickness 11.98 µm followed by 11.44 µm in Dh-22 (Red) and K-134 (10.32 µm). However, susceptible genotype KRG-1 recorded significantly lesser cuticular (3.06 µm) and epidermal cell thickness (9.02 µm). The results on epidermal cell number in these genotypes followed reverse pattern. Resistant and moderately resistant genotypes showed significantly minimum epidermal number compared to susceptible ones. Among them, GPBD-4 recorded lesser number of epidermal cells (32.01 cells/mm) followed by Dh-22 (Red) (33.12 cells/mm) while, R-8808 and K-134 measured 34.12 µm.
It was apparent from the present investigation that less number of epidermal cells per mm and increased epidermal cell layer thickness occur in resistant and moderately resistant genotypes than susceptible genotypes. These act as first line of defense to prevent invasion of pathogen. These results are in agreement with the findings of Mayee and Apet (1995) who reported that, genotypes of groundnut resistant to leaf rust had thicker epidermis cum cuticle. Thus, thicker epidermis cum cuticle along with lesser epidermal cell number act as the physical barrier for the entry of the pathogen imparting resistance to genotypes.

**Number and size (length, breadth) of stomata**

The data presented in Table 1 revealed that resistant and moderately resistant genotypes showed lesser number and size (length, breadth) of stomata compared to susceptible genotypes on both adaxial and abaxial leaf surfaces. Among them, least stomatal number (8.50/mm²) was found in GPBD-4 and Dh-22 (Red) followed by K-134 (12.75/mm²) and R-8808 (13.00/mm²) on adaxial surface. However, susceptible genotypes KRG-1 (16.75/mm²) and TMV-2 (16.00/mm²) recorded more stomatal number. The analysis of stomatal frequency on abaxial surface also revealed the same trend of results as observed in case of adaxial surface. Hence, it was found that stomatal number was comparatively more in abaxial leaf surface than on adaxial leaf surface in all genotypes. The results on size of stomata revealed that, both resistant and moderately resistant genotypes recorded lesser length and breadth of stomata when compared to susceptible genotypes on both adaxial and abaxial leaf surfaces. Further, results indicated that, length and breadth of stomata were found less on abaxial leaf surface than adaxial.

It can be emphasized that the stomatal frequency was higher in susceptible genotypes. More number of stomata were recorded on abaxial surface than that of adaxial surface of leaf which indicates that, these act as main avenue for the entry of the pathogen into leaf tissue. The size (length, breadth) of stomata was also more in susceptible genotypes as compared to resistant and moderately resistant genotypes. Susceptible genotypes recorded higher frequency and size of stomata which provides higher opportunity for penetration by these pathogens and results in high disease severity than resistant ones. These results are in agreement with studies conducted by different workers (Mayee and Apet, 1995 and Benagi, 1995). They reported that, in crop like groundnut the frequency and size of stomata were significantly lower on abaxial surface of leaves of resistant genotypes against leaf spot and rust diseases. Further, they reported that the number and size of the stomata are important characters of the leaf in relation to resistance or susceptibility of the plant to many foliar pathogens.

**Wax content**

The wax content varied significantly among the genotypes at different crop growth stages (Table 2). Wax content was increased from 25 DAS to 75 DAS in resistant and moderately resistant genotypes. Whereas, it was decreased with increase in age of the crop in susceptible genotypes.

### Table 1. Frequency and size of stomata in resistant, moderately resistant and susceptible varieties of groundnut to rust caused by *P. arachidis*

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Variety</th>
<th>Number of stomata (mm²)</th>
<th>Size of stomata</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Adaxial surface</td>
<td>Abaxial surface</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Length (µm)</td>
<td>Breadth (µm)</td>
</tr>
<tr>
<td>1</td>
<td>Dh-22 (Red)</td>
<td>8.50</td>
<td>11.00</td>
</tr>
<tr>
<td>2</td>
<td>GPBD-4</td>
<td>8.50</td>
<td>10.86</td>
</tr>
<tr>
<td>3</td>
<td>K-134</td>
<td>12.75</td>
<td>11.75</td>
</tr>
<tr>
<td>4</td>
<td>R-8808</td>
<td>13.00</td>
<td>12.86</td>
</tr>
<tr>
<td>5</td>
<td>KRG-1</td>
<td>16.75</td>
<td>16.00</td>
</tr>
<tr>
<td>6</td>
<td>TMV-2</td>
<td>16.00</td>
<td>16.21</td>
</tr>
<tr>
<td>S.Em±</td>
<td>0.64</td>
<td>0.82</td>
<td>1.31</td>
</tr>
</tbody>
</table>

| CD at 1% | 2.56 | 3.21 | 1.31 | 2.95 | 3.13 | 4.65 |
The resistant variety, GPBD-4 recorded maximum wax content of 0.45 mg/dm² at 25 DAS, increased to 0.85 mg/dm² at 50 DAS and reached as high as 0.95 mg/dm² at 75 DAS. The variety Dh-22 (Red) was next best in recording more wax as it recorded the range of 0.46 to 0.91 mg/dm² at 25 and 75 DAS, respectively. The least wax content of 0.16 mg/dm² was observed in KRG-1 at 25 DAS and it was further decreased to 0.12 mg/dm² at 50 DAS to the least of 0.07 mg/dm² at 75 DAS.

The natural protective covering of the epidermal cells of leaves of groundnut consists of the cuticle with its waxy coating and wettability of leaves is related to early infection process of *P. arachidis*. More amount of wax on leaf surface leaf lamina could not be easily wetted as wax is negatively charged particle. Thus, wax content of leaf is one of the important parameters of resistance. It was apparent from the data that resistant genotypes viz., GPBD-4 and Dh-22 (Red) showed maximum wax content in addition to the increased wax content as age of the genotypes advanced from 25 to 75 DAS. While, the least wax content was observed in susceptible genotypes, KRG-1 and TMV-2 it was decreased further as the age advanced from 25 to 75 DAS. Similar results have been reported by Benagi (1995) who reported that, the wax content was least in susceptible genotypes than that of resistant variety of groundnut infected by *C. arachidicola*.

### Biochemical mechanisms

#### Sugars

Total sugar, reducing sugar and non-reducing sugar content were more in diseased leaves compared to healthy leaves. Further, resistant and moderately resistant genotypes recorded more sugars than susceptible ones. In the present investigation, diseased leaves of different genotypes showed more total, reducing and non-reducing sugar content compared to healthy leaves (Table 3). Further, when we compared sugar content within genotypes, the sugars were more in resistant and moderately resistant genotypes than susceptible ones which were in confirmation with the findings of Ekbote and Mayee (1983).

### Total phenol and ortho-dihydroxy phenol

More total phenol and ortho-dihydroxy phenol contents (Table 3) were recorded in healthy leaves than in diseased ones. The resistant genotypes viz., GPBD-4 and Dh-22 (Red) recorded highest phenol content of 1.61 and 1.46 mg/dry wt respectively. While corresponding diseased leaves of same genotypes contained less phenol content (0.66 and 0.65 mg/dry wt.). Similarly, healthy leaves showed more ortho-dihydroxy phenol content when compared to diseased leaves. The highest content was observed in GPBD-4 (1.00 m/dry wt) followed by Dh-22 (Red) (0.98 mg/dry wt). The diseased leaves of same genotypes recorded lesser content of 0.83 and 0.91 mg/dry wt., respectively. Similar results were also reported by Reddy and Ravindranath (1988); Velazahan and Vidyasekharan (1994); Kumar and Balasubramanyan (2000).

### Protein

The highest protein content of 6.68 mg/dry wt. was recorded in the healthy leaves of GPBD-4 followed by 6.47 mg/dry wt. in Dh-22 (Red) and R-8808 (4.81 mg/dry wt.). However, the diseased

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**Table 2.** Changes in wax content in leaves of resistant, moderately resistant and susceptible varieties influenced by rust of groundnut caused by *P. arachidis*

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Variety</th>
<th>Percent Disease Index (PDI)</th>
<th>Wax content (mg / dm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25 DAS</td>
<td>50 DAS</td>
</tr>
<tr>
<td>1</td>
<td>Dh-22 (Red)</td>
<td>0</td>
<td>12.22</td>
</tr>
<tr>
<td>2</td>
<td>GPBD-4</td>
<td>0</td>
<td>14.81</td>
</tr>
<tr>
<td>3</td>
<td>K-134</td>
<td>0</td>
<td>14.81</td>
</tr>
<tr>
<td>4</td>
<td>R-8808</td>
<td>0</td>
<td>16.30</td>
</tr>
<tr>
<td>5</td>
<td>KRG-1</td>
<td>0</td>
<td>19.63</td>
</tr>
<tr>
<td>6</td>
<td>TMV-2</td>
<td>0</td>
<td>19.63</td>
</tr>
<tr>
<td></td>
<td>S.Em±</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD at 1%</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

S.Em± - - - 0.05 0.06 0.08
CD at 1% - - - 0.24 0.24 0.35
leaves of corresponding genotypes recorded lesser content of protein (Table 3). The protein biosynthesis of the host is widely assumed to be significant feature of pathogenesis, particularly during incompatible reaction. Quantitatively speaking, the total protein synthesis is much enhanced in the healthy tissues around the infected tissues. This additional protein considered to be entirely of host origin (Dasgupta, 1988). In the present findings healthy leaves of both resistant and moderately resistant genotypes recorded more of soluble protein than the diseased leaves of susceptible ones. These are in agreement with Patel and Vaishnav (1986); Narayan Reddy and Khare (1988) in groundnut rust.

REFERENCES


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