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Morphological and Biochemical Characterization of *Phaeoisariopsis personata* Resistant and Susceptible Cultivars of Groundnut (*Arachis hypogaea*)

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ABSTRACT

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Thirteen groundnut (*Arachis hypogaea*) cultivars were screened for resistance to *Phaeoisariopsis personata* under natural conditions. They were classified into resistant (cultivar FDRS-10), moderately resistant (cultivars K - 134, TCGS-l56 and Tpt-3) and susceptible (Tpt-l, Tpt-2, TCGS-29, TCGS-91, TCGS-l50, TCGS-341, Tpt-4 JL-24 and TMV-2), based on a disease severity of 1-9 scale. Morphological characters namely stomatal frequency and size, biochemical parameters namely chlorophyll, total phenols, peroxidase and polyphenol oxidase were estimated among the cultivars having different degree of resistance. The stomatal frequency and size was less in resistant cultivar, FDRS-10 and more in susceptible cultivar, TMV-2. The chlorophyll content was highest in resistant cultivar, which decreased upon infection in all cultivars. Total phenols, peroxidase and polyphenol oxidase were higher in resistant cultivar, FDRS-10 and upon infection, increased further, in all cultivars under study. Significant differences in banding profiles of phosphatase and esterase isozymes were observed in all cultivars. One phosphatase band at Em of 0.07 and two esterase bands at Em of 0.50 and 0.63 were specific to resistant cultivar, FDRS-l0 only. The detected isozyme variability could possibly be used as biochemical marker in identification of *Phaeoisariopsis personata* resistant cultivars of groundnut.

Key words : Groundnut, late leaf spot, *Phaeoisariopsis personata*, biochemical resistance, isozyme analysis

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the important oil seed crops of India, growing in the semi-arid tropics. Diseases of groundnut reduce yield and quality and increase the cost of production wherever the crop is grown [(28)]. Among the groundnut diseases, late leaf spot (LLS) caused by *Phaeoisariopsis personata* (Berk. & Curt.) V. Arx. 〔= *Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton〕 and rust caused by *Puccinia arachidis* Speg. are the most serious fungal diseases worldwide and then account for more than 50% yield losses [(23)]. The late leaf spot disease generally appears at 55 to 60 days after sowing and can cause more than 50% loss in pod and haulm yields in groundnut producing areas of Karnataka [(10)]. Reduction in pod and haulm yield was reported as 25.3% 53.0%, respectively [(7)]. The existing fungicidal control measures available are not economical apart from causing environmental pollution. Hence, an economic and eco-friendly way to manage the disease effectively is through host plant resistance. Several workers have reported partial resistance in cultivated groundnuts [(26)]. It was reported that among 28 groundnut genotypes screened for resistance to late leaf spot and rust, only 5 were found to be resistant, 3 genotypes moderately resistant [(4)].

Several physical barriers present either on the leaf surface or in the tissues of plant make it difficult for entry or spread of the pathogen within the host. It is expected that different genotypes may differ in their response to infection by *P. personata*. An analysis of changes in biochemical constituents of healthy and diseased leaves of resistant and susceptible cultivars would help in understanding the biochemical defense mechanism. In order to identify the

resistant cultivars based on biochemical markers, poly acrylamide gel electrophoresis (PAGE) of phosphatase and esterase was carried out. Since information on isozyme variability among groundnut cultivars is scanty, the objectives of this study were (1) To isolate the pathogen from the infected leaves, identify and test its pathogenecity on groundnut; (2) To determine resistance of groundnut cultivars to *Phaeoisariopsis personata*; (3) To compare morphological characters namely stomatal frequency and size in different groundnut cultivars; (4) To determine biochemical constituents namely chlorophyll content, total phenols, peroxidase and polyphenol oxidase in different groundnut cultivars; and (5) To determine the isozyme variability among groundnut cultivars.

MATERIALS AND METHODS

Screening groundnut cultivars for resistance to late leaf spot

Thirteen groundnut cultivars, namely Tpt-l, Tpt-2, Tpt-3, Tpt-4, TCGS-29, TCGS-91, TCGS-150, TCGS-I56, TCGS-341, FDRS-10, JL-24, K-134 and TMV-2, were obtained from Regional Agricultural Research Station (RARS), Tirupati, Andhra Pradesh, India and were used in the present study.

The experiment was conducted in a field naturally infested with *P. personata* during rainy season (June-October, 2002) at Sri Venkateswara Agricultural College Wetland Farm, Tirupati, Andhra Pradesh in plots of size 2m x 2m (l x w) in randomized block design along with recommended dosage of fertilizers (20, 50, 40 kg N, P, K, respectively per hectare applied in 2 splits one at sowing and the other at 30 days after sowing; 500 kg gypsum per hectare applied during peg formation stage) and replicated three times.

The Percent Disease severity Index (PDI) was calculated on leaf lesions of different groundnut cultivars at the time of harvest using the following formula.

$$PDI = \frac{\text{Sum of all ratings}}{\text{Number of leaves scored}} \times \frac{100}{\text{Maximum score}}$$

For the estimation of disease incidence, fifty leaves were collected from each plot in each treatment and the disease was recorded using modified 9-point scale [24] as given below:

1 = No disease; 2 = 1 to 5% disease severity; 3 = 6 to 10% disease severity; 4 = 11 to 20% disease severity; 5 = 21 to 30% disease severity; 6 = 31 to 40% disease severity; 7 = 41 to 60% disease severity; 8 = 61 to 80% disease severity; 9 = 81 to 100% disease severity.

Determination of stomatal frequency and size

The mounted epidermal impressions were placed under microscope and the stomatal frequency (number of stomata) was counted within the high power field of microscope (10x x 40x). Twenty leaves were collected randomly from each plot and countings were taken at three randomly selected areas of each leaf and average number of stomata per microscopic field was counted. Based on this data, the number of stomata per mm^2 of leaf surface was calculated.

The size of stomata was determined using micrometry technique. The ocular micrometer was first calibrated using stage micrometer. The length and width of the stomatal aperture was measured with the help of calibrated ocular micrometer.

Estimation of chlorophyll

Chlorophyll estimation of healthy and inoculated plants was done at 30 days after inoculation [1]. Third leaf from the apex of groundnut plants was chosen in all genotypes and 20 such leaves were randomly collected per plot for chlorophyll estimation. The following formulae were used for estimation of total chlorophyll, chlorophyll 'a' and chlorophyll 'b' and the results were expressed as mg of chlorophyll/ g of fresh weight.

Total chlorophyll: (20.2 x OD at 645 nm) + (8.02 x OD at 663 nm) df
Chlorophyll 'a': (12.7 x OD at 663 nm) -(2.69 x OD at 645 nm) df.
Chlorophyll 'b': (22.9 x OD at 645 nm) -(4.68 x OD at 663 nm) df.
Where, OD = Optical Density; df = dilution factor.

Estimation of phenols

Total phenol content in the leaves of healthy and inoculated groundnut cultivars namely TMV-2, TCGS-29, K-134, FDRS-10 were estimated at 5, 10, 15 and 20 days after inoculation by following procedure of [19]. The results were expressed by measuring absorbance at 650 nm using spectronic-20 D (Milton Roy) in mg per 100 g of tissue.

Quantification of peroxidase and poly phenol oxidase activity

The procedure of peroxidase detection[8] was adopted for determining the activity of peroxidase and poly phenol oxidase. Activity of these enzymes was determined from selected healthy and inoculated cultivars of TMV-2, TCGS-29, K-134, FDRS-10 at 2, 4, 6, 8, 10 and 12 days after inoculation.

Peroxidase activity was measured by change in absorbance at 430 nm at 15 seconds interval from zero time for 3 min using Spectronic-20. Similarly, poly phenol oxidase activity was measured by change in absorbance at 400 nm at an interval of 15 seconds from zero time of reaction using Spectronic- 20 D (Milton Roy).

Specific activity of the enzyme was calculated with the following formula.

$$\text{Specific activity} = \frac{\text{Enzyme protein}}{\text{mg protein}} = \frac{\triangle E\ 1000V}{\text{t. mg protein}}$$

Where, $\triangle E$= Extinction co-efficient; V = volume of the reaction mixture; and t = time in seconds.

Analysis of phosphatase and esterase isozyme by Poly Acrylamide Gel Electrophoresis (PAGE)

Detection of phosphatase: The leaves from cultivars, TMV-2, Tpt-2, Tpt-1, TCGS-29, TCGS-91, TCGS-150, FDRS-10, K-134 and TCGS-156 were collected and isozyme was extracted [21]. 70 μl of sample was loaded and electrophoresis was carried out at a constant current of 30 mA at 4℃. The experiment was terminated when the dye reached the bottom of the gel. After electrophoresis, the gel was incubated in the dark at 30℃ for 2 h in a solution of 50 mM sodium acetate buffer (pH 5.5) containing 1.0 M magnesium chloride, 0.1% Fast Garnet GBC salt and 1% α - naphthyl acid phosphate until the red bands appeared. After the reaction, the gel was preserved in distilled water.

Detection of esterase: The leaves from cultivars, Tpt-3, TCGS-341, TCGS-150, TCGS-91, TCGS-156, K-134, FDRS-10, Tpt-2, Tpt-1, JL-24, and TMV-2 were used for isozyme extraction. 70 μl of extracted sample was loaded into gel and electrophoresis was carried out at a constant current of 30 mA at 4℃. The experiment was terminated when the dye reached the bottom of the gel. After electrophoresis, the gel was incubated for 30-45 min, in a solution of 0.1M sodium phosphate buffer (pH 7.2) containing 0.1% Fast Blue RR salt and 0.4% α - naphthyl acetate. The gel was destained with water : methanol : acetic acid (15 : 4 : 1 V/V/V).

The electrophoretic mobility (Em) for isozyme band was calculated and zymograms were constructed. The Em of band was calculated as:

$$Em = \frac{\text{Distance traveled by the band}}{\text{Distance traveled by the dye front}}$$

Data analyses

The data pertaining to field experiment was analyzed using randomized block design and all the data collected in lab was analyzed using completely randomized design. Analysis of variance was carried out to determine significant differences among cultivars in per cent disease severity, pod and haulm yield, stomatal number and size, chlorophyll content, total phenols, peroxidase and poly phenol oxidase activity. All statistical analyses were conducted using M-STAT software.

RESULTS

Pathogenecity test

The pathogenecity test produced leaf spots first recognized as small chlorotic specks, which enlarged later and became light to dark brown lesions. The lesions were smaller, circular and darker. They were black and slightly rough in appearance on the lower surface. These spots later spread to petioles and stems (Fig. 1).

Fig. 1. Black lesions observed on the groundnut leaves affected with late leaf spot. Left : Healthy leaf; Right: late leaf spot affected leaf.

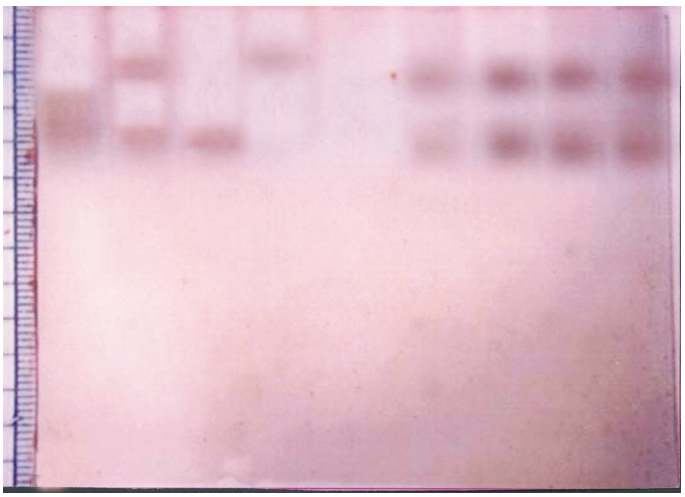

Fig. 2. Phosphatase isozyme banding pattern in selected groundnut cultivars. Lane 1: TMV-2; Lane 2: Tpt-2; Lane3: Tpt-1; Lane 4: TCGS-29; Lane 5: TCGS-91; Lane 6: TCGS-150; Lane 7: FDRS-10; Lane 8: K-134; Lane 9: TCGS-156.

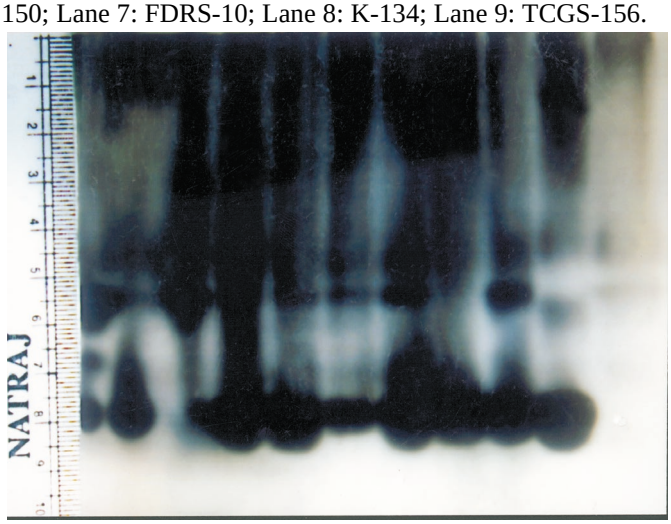

Fig. 3. Esterase banding pattern observed in selected groundnut cultivars. Lane 1: Tpt-3; Lane 2: TCGS-341; Lane 3: TCGS-150; Lane 4: TCGS-91; Lane 5:TCGS-156; Lane 6: K-134; Lane 7: FDRS-10; Lane 8: Tpt-2; Lane 9: Tpt-1; Lane 10: Jl-24; Lane 11: TMV-2.

Table 1. Late leaf spot disease severity, pod and haulm yields among different groundnut cultivars under natural conditions.

Cultivar	PDI[2] (%)	Disease Rating[3]	Pod yield (g/plot)	Haulm yield (kg/plot)
Tpt-2	58.4 (49.8) [1]	S	426.7	3.4
Tpt-1	59.7 (50.6)	S	665.0	3.4
TCGS-29	49.7 (43.7)	S	645.0	3.3
Tpt-3	35.7 (36.7)	MR	406.7	4.1
FDRS-10	9.9 (18.3)	R	591.7	3.4
TCGS-91	48.6 (44.2)	S	763.3	4.3
TCGS-150	52.8 (46.6)	S	546.7	3.6
TCGS-156	25.0 (30.0)	MR	626.7	3.2
Tpt-4	50.9 (45.5)	S	723.3	3.4
TCGS-341	41.4 (40.1)	S	653.3	3.7
K-134	23.3 (28.3)	MR	561.7	2.9
TMV-2	64.4 (53.4)	S	496.7	3.0
JL-24	61.5 (51.7)	S	446.7	2.5
SD (P=0.05)	0.6		0.7	0.2

[1] Figures in parantheses are angular transformed values.
[2] Mean of three replications.
[3] Disease Rating : S=Susceptible; MR=Moderately Resistant; R=Resistant.

Table 2. Stomatal frequency (No. of stomata/mm^2) and size in different groundnut cultivars at 30 DAI.

Cultivar	Stomatal frequency[2] (per mm^2)	Stomatal size[2] Length of stomata (μm)	Breadth of stomata (μm)	PDI (%)
Tpt-2	179.8	24.8	11.0	58.3 (49.8)[1]
Tpt-1	190.1	26.1	11.0	59.7 (50.6)
TCGS-29	161.6	23.1	7.6	49.7 (43.7)
Tpt-3	148.7	22.0	6.1	35.7 (36.7)
FDRS-10	129.9	19.3	5.8	9.98 (18.3)
TCGS-91	157.5	23.0	6.9	48.6 (44.2)
TCGS-150	175.4	23.8	8.6	52.8 (46.6)
TCGS-156	143.3	22.0	7.3	25.0 (30.0)
Tpt-4	166.5	23.6	8.3	50.9 (45.5)
TCGS-341	156.9	22.9	7.0	41.4 (40.1)
K-134	134.8	20.3	6.6	23.3 (28.3)
TMV-2	195.2	29.3	11.7	64.4 (53.3)
JL-24	194.8	28.2	11.3	61.5 (51.7)
± SD (P=0.05)	21.92	2.87	2.11	

[1] Figures in parantheses are angular transformed values.
[2] Mean of three replications.

Table 3. Chlorophyll content of healthy and inoculated groundnut cultivars at 45 DAI.

Cultivars	Healthy (mg/ g)[1] Chl. a	Chl. b	Total chlorophyll	Inoculated (mg/g) Chl. a	Chl. b	Total chlorophyll
Tpt-2	0.6	0.4	1.0	0.4	0.2	0.6
Tpt-1	0.5	0.2	0.7	0.4	0.2	0.5
TCGS-29	0.7	0.5	1.2	0.6	0.4	1.0
Tpt-3	0.8	0.5	1.3	0.7	0.5	1.2
FDRS-10	1.0	0.5	1.5	0.9	0.5	1.4
TCGS-91	0.8	0.4	1.2	0.7	0.4	1.1
TCGS-150	0.6	0.5	1.1	0.6	0.3	0.8
TCGS-156	0.9	0.5	1.3	0.8	0.5	1.3
Tpt-4	0.7	0.4	1.1	0.5	0.4	0.9
TCGS-341	0.9	0.4	1.3	0.7	0.5	1.2
K-134	0.9	0.5	1.4	0.9	0.4	1.3
TMV-2	0.4	0.3	0.6	0.2	0.1	0.3
JL-24	0.4	0.2	0.7	0.2	0.2	0.4
± SD (P=0.05)	0.2	0.1	0.3	0.2	0.1	0.3

[1] Third leaf from apex was taken for chlorophyll estimation and chlorophyll content expressed as mg chlorophyll/g of fresh weight of leaf tissue.

Table 4. Total phenol content in healthy and inoculated leaves of selected groundnut cultivars at different days after inoculation.

Cultivar	Treatment	Total phenols in fresh leaf (mg / 100 mg) at DAI[1] 5 th	10 th	15 th	20 th	Mean
TMV-2	Healthy	2.5	3.0	4.1	4.9	3.6
	Inoculated	2.7	3.9	5.1	6.1	4.4
		(6.4)	(28.7)	(24.8)	(25.3)	
TCGS-29	Healthy	2.6	3.5	4.5	5.2	4.0
	Inoculated	3.1	4.4	6.1	7.0	5.1
		(20.3)	(25.2)	(33.6)	(35.4)	
K-134	Healthy	2.8	3.7	4.9	5.3	4.1
	Inoculated	3.3	4.7	6.5	7.3	5.5
		(21.0)	(29.1)	(32.7)	(38.8)	
FDRS-10	Healthy	3.4	4.1	5.1	6.2	4.7
	Inoculated	4.2	5.3	7.0	8.8	6.3
		(24.2)	(30.6)	(36.0)	(41.6)	
Mean		3.1	4.1	5.5	6.4	
± SD (P=0.05)		0.6	0.7	0.9	1.3	

[1] DAI: Days after inoculation.
[2] Figures in parenthesis refer to percentage increase in total phenol content of inoculated groundnut leaves.

Screening groundnut cultivars for resistance

The data on late leaf spot severity in different groundnut cultivars and disease reaction is presented in Table 1. These results revealed that the severity of the late leaf spot was 10.0% for cultivar FDRS-10 and 23.3% for cultivar K-134. Highest disease severity was observed in case of cultivar TMV-2 with a PDI of 64.4%.

The cultivars were classified into three groups based on the PDI: 1. Resistant (1 to 10%): FDRS-10; 2. Moderately resistant (11 to 40%): K-134, TCGS-156 and Tpt-3; and 3. Susceptible (41 to 100%): TMV-2, JL-24, Tpt-2, Tpt-l, TCGS- 29, TCGS-91, Tpt-4 and TCGS-341.

Stomatal frequency and size

There were significant differences among groundnut cultivars with regard to stomatal frequency and size. The stomatal frequency ranged from 129.9 per mm^2 in the resistant cultivar FDRS-10 to 195.2 per mm^2 in susceptible

Table 5. Peroxidase activity in leaves of selected groundnut cultivars at different days after inoculation (DAI).

Cultivar	Treatment	Leaf peroxidase activity (Specific weight / enzyme protein) at DAI[1]						Mean
		2 nd	4 th	6 th	8 th	10 th	12 th	
TMV-2	Healthy	1.0	1.1	1.3	1.2	1.3	1.3	1.2
	Inoculated	1.3	1.4	1.7	1.8	1.7	1.7	1.6
		(23.3)	(26.6)	(35.2)	(51.3)	(3.1)	(34.4)	
TCGS-29	Healthy	1.2	1.2	1.3	1.3	1.4	1.4	1.3
	Inoculated	1.5	1.6	1.8	1.9	1.8	1.7	1.7
		(25.6)	(32.3)	(35.9)	(52.0)	(30.9)	(26.5)	
K-134	Healthy	1.3	1.4	1.6	1.5	1.4	1.3	1.4
	Inoculated	1.6	1.9	2.1	2.3	2.0	1.8	2.0
		(23.9)	(36.2)	(36.5)	(53.4)	(43.9)	(40.6)	
FDRS-10	Healthy	1.4	1.5	1.6	1.6	1.5	1.4	1.5
	Inoculated	1.7	1.9	2.2	2.5	2.2	2.0	2.1
		(25.9)	(31.3)	(32.5)	(57.6)	(44.7)	(42.5)	
Mean		1.4	1.5	1.7	1.8	1.7	1.6	
± SD (P=0.05)		0.2	0.3	0.3	0.4	0.3	0.3	

[1] DAI: Days after inoculation.
[2] Figures in parenthesis refer to percentage increase in peroxidase activity of inoculated groundnut leaves.

Table 6. Polyphenol oxidase activity in leaves of selected groundnut cultivars at different days after inoculation (DAI).

Cultivar	Treatment	Leaf polyphenol oxidase activity (Specific weight / enzyme protein) at DAI[1]						Mean
		2 nd	4 th	6 th	8 th	10 th	12 th	
TMV-2	Healthy	1.3	1.4	1.6	1.5	1.4	1.3	1.4
	Inoculated	1.5	1.7	2.0	2.2	2.0	1.7	1.9
		(15.6)	(18.1)	(20.9)	(46.7)	(42.9)	(38.4)	
TCGS-29	Healthy	1.4	1.5	1.7	1.6	1.5	1.4	1.5
	Inoculated	1.6	1.8	2.1	2.4	2.2	2.0	2.0
		(17.0)	(18.2)	(24.7)	(48.5)	(6.0)	(42.9)	
K-134	Healthy	1.4	1.6	1.9	1.7	1.5	1.5	1.6
	Inoculated	1.7	2.0	2.4	2.7	2.3	2.1	2.2
		(19.2)	(22.6)	(27.0)	(54.9)	(48.0)	(44.8)	
FDRS-10	Healthy	1.8	1.9	2.1	2.0	1.9	1.6	1.9
	Inoculated	2.1	2.4	2.7	3.1	2.9	2.4	2.4
		(21.1)	(23.6)	(28.1)	(56.0)	(50.8)	(47.2)	
Mean		1.6	1.8	2.1	2.2	1.9	1.8	
± SD (P=0.05)		0.3	0.3	0.3	0.5	0.5	0.4	

[1] DAI: Days after inoculation.
[2] Figures in parenthesis refer to percentage increase in polyphenol oxidase activity of inoculated groundnut leaves.

cultivar TMV-2 (Table 2). The size of stomata in FDRS-10 was 19.25 x 5.84 μm whereas in TMV-2 it was 19.25 x 11.69 μm (Table 2).

Biochemical characterization

Estimation of chlorophyll : Total chlorophyll content among the healthy groundnut cultivars ranged from 0.6 mg/g to 1.5 mg/g. In inoculated groundnut cultivars, the chlorophyll content decreased, which ranged from 0.3 to 1.4 mg/g. The chlorophyll content was high in resistant cultivar and low in susceptible groundnut cultivars. There was significant difference between healthy and inoculated leaves of the respective groundnut cultivars (Table 3).

Estimation of total phenols : Highest phenol content was recorded in resistant cultivar, FDRS-10 (4.7 mg/100 mg fresh weight), as against susceptible cultivar, TMV-2 which recorded 3.6 mg/100 mg fresh weight (Table 4).

In inoculated and diseased plants, a significant increase in total phenol was observed in resistant cultivar, FDRS-10 which is equivalent to 41.6% increase at 20 days after inoculation. A significant increase of 28.7% was observed at 10 days after inoculation in susceptible cultivar, TMV-2 (Table 4).

Quantification of peroxidase and polyphenol oxidase activity

The leaf peroxidase activity recorded in both healthy and inoculated groundnut cultivars was presented in Table 5. In

healthy leaves, higher peroxidase activity was recorded in FDRS-10 (1.5 specific activity/enzyme protein) followed by K-134 (1.4 specific activity/enzyme protein) when compared to susceptible cultivar, TMV-2 (1.2 specific activity/enzyme protein). On infection, a significant change was observed in all cultivars, maximum increase compared to control being in FDRS-10 (57.6%) and minimum increase in TMV-2 (51.3%) at 8 DAI.

Higher poly oxidase phenol activity was observed in FDRS-10 (1.9 specific activity/enzyme protein) and least in TMV-2 (1.4 specific activity/enzyme protein). Upon infection, significant difference was observed in all cultivars, maximum increase compared to control being in FDRS-10 (56%) at 8 days after inoculation, which later on decreased (Table 6).

Isozyme characterization

Phosphatase : The isozyme profiles of seed phosphatase indicated a total of two bands among cultivars. A strong band at Em of 0.24 was observed in cultivars FDRS-10, K-134, TCGS-156 and TMV-2, less represented in Tpt-l, Tpt-2 and TCGS-150 and absent in TCGS-29 and TCGS-91. Another band at 0.07 Em was observed with different intensities in almost all cultivars except in TMV-2, Tpt-l and TCGS-91. However, in cultivar TCGS-91, all these bands were absent (Fig. 2).

Esterase : The isozyme profiles of esterase revealed the presence of a major band at Em 0.68 in all cultivars except in TCGS-150, JL-24 and TMV-2. Additional bands at Em of 0.63, 0.50 and 0.45 were observed only in resistant cultivars FDRS-10 and K-134. Two medium intense bands at Em of 0.54 and 0.40 were observed in JL-24 and at Em of 0.45 and 0.40 were observed in TMV-2 (Fig. 3).

DISCUSSION

Pathogenecity test of *P. personata* on groundnut carried at 30 days after sowing under pot culture conditions showed leaf spot symptoms at 65 days after sowing. Lesions formed by *P. personata* tend to be smaller, more nearly circular and darker. On the lower surface, the lesions are black and slightly rough as the fungus sporulates on the lower surface. Similar types of symptoms were described by [15, 17].

Frequent application of fungicides may lead to development of tolerance in the target organism [22]. Genotypes resistant to the disease would be cheap and stable management practice [27]. In the present study, FDRS-10 was found to be resistant, K-134 as moderately resistant and TMV-2 as most susceptible cultivar.

It was reported that none of the groundnut cultivars showed immune reaction to both late leaf spot and rust, whereas 20 cultivars were tolerant, 7 moderately susceptible and 5 susceptible [6]. The high yielding susceptible cultivars can be crossed with comparatively low yielding late leaf spot resistance lines such as FDRS-10, K-134 to combine higher resistance with an improved yield. The lines developed through hybridization had moderate levels of resistance with high yield [28].

Certain pre-existing structural characteristics are present to defend the plant against invasion or attack by pathogen. Significant differences in stomatal frequency and size were observed between resistant and susceptible cultivars. The resistant cultivar FDRS-10 has shown less number and size compared to susceptible, TMV-2.

Resistant genotypes are characterized by smaller sized stomata of low frequency as reported [14, 25] with groundnut and *P. personata* system. Thickness of epidermis, low stomatal index, smaller size of stomata and high palisade index were extremely useful in the defence mechanism of groundnut to late leaf spot pathogen [2, 13].

The biochemical dynamics of parasitism and pathogenesis are triggered and controlled by a series of interactions between host and pathogen. Most of the research on disease resistance has shown that the plant uses its defense mechanism that is activated after infection to stop pathogen development in the host. Phenols have long been associated with passive and active defense responses of plants. In the present study, biochemical parameters such as chlorophyll content, total phenols, and quantitative assay of peroxidase and poly phenol oxidase were carried out. Significant differences in chlorophyll content of all cultivars were observed which upon infection decreased. In the present study, the decrease in total chlorophyll, chlorophyll 'a' and chlorophyll 'b' due to late leaf spot infection was more pronounced in susceptible cultivars than the resistant ones [3].

The present study also revealed that the leaf total phenols varied with cultivar. In healthy cultivars, higher quantities of phenols were observed in resistant cultivar, FDRS-10 and least in susceptible cultivar, TMV-2. In inoculated leaves of all cultivars, quantity of phenols progressively increased on all the days, highest being on 20 days after inoculation. The post infectional increase in phenolic contents could be due to enhancement of synthesis, translocation of phenolics to the site of infection and hydrolysis of phenolic glycosides by fungal glycosidase to yield free phenols [20]. Increase in phenols after infection was also observed in banana infected by *Cercospora musae* and *Helminthosporium gibberosporium* [11].

Pathogenic infections of host plants after leading to alterations in the enzyme systems of the plants i.e. changes in the oxidases, catalase etc. of infected plants have been reported by several investigators. In the present study, greatest increase of peroxidase and poly phenol oxidase activity was observed at 8 DAI in all cultivars. Increase in peroxidase and poly phenol oxidase activity upto 8 DAI and then decrease was observed in case of *Puccinia arachidis* affected groundnut leaves [16]. Increased activity of peroxidase upon

infection might be essential for an additional deposition of lignin around the lesions induced by pathogens. The increased activity of poly phenol oxidase was reported due to either solubilization of polyphenolases from cellular compartments or activation of latent polyphenol oxidase [18]. Similar increase in both poly phenol oxidase and peroxidase enzymes following infection has also been reported in other host parasite combinations [1,9].

The detected variability in isozyme pattern suggests the existence of genetic variability among the resistant and susceptible cultivars. Thus, the polymorphism observed in leaf phosphatases and esterases could be detected and used as biochemical markers in identifying the late leaf spot resistant cultivars after screening more number of genotypes.

In conclusion, the application of these techniques either alone or in combination could help in identification and characterization of various cultivars posessing degree of late leaf spot resistance. The experiments are in progress to make a cross between resistant and susceptible cultivars. Thus obtained progeny will be screened for late leaf spot and subjected to isozyme and nucleic acid based marker analysis in order to link the late leaf spot resistance traits.

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摘 要

Jyosthna, M. K.[1], Eswara Reddy, N. P.[1,3], Chalam, T. V.[1], and Reddy, G. L. K.[2] 2004. 落花生黑澀病抗感病品種的形態與生化特性. 植病會刊13：243-250. ([1.] 印度 Sri Venkateswara 大學農學院植物病理學系；[2.] 印度 Bapatla 農學院基因與植物育種學系；[3.] 聯絡作者，電子郵件：eswarnp@yahoo.com；傳眞：+0091-877-2248667)

在自然狀況下，篩選 13 個落花生品種對黑澀病的抗感病性，依1-9 級罹病度可將受測品種分成抗病 (FDRS-10 品種)、中抗 (K-134、TCGS-156 和Tpt-3三個品種) 及感病 (Tpt-1、Tpt-2 、TCGS-29、TCGS-91、TCGS-150、TCGS-341 、Tpt-4、JL-24 和TMV-2 等品種) 等三群。比較各品種間的形態特徵如單位氣孔數及大小與生化特性如葉綠素、全酚化物、過氧化酵素和多酚氧化酵素等活性。在抗病品種 FDRS-10 葉片上，其氣孔數及大小較少，而感病品種 TMV-2 則較多。植株受感染後，抗病品種的葉綠素含量、全酚化物、過氧化酵素和多酚氧化酵素活性均較其他品種爲高。另外，以同分異構酶分析品種間磷酸化酵素及酯化酵素，結果只在抗病品種 FDRS-10 上發現位於 0.07 Em 處有一條磷酸化酵素條帶及於 0.50 及 0.63 Em 處發現二條酯化酵素條帶。同分異構酶似可作爲鑑定落花生品種抗黑澀病的生化標定物。

關鍵詞：落花生、黑澀病 、同分異構酶分析、生化抗病性