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Studies on Pre-Existing Morphological Defense Structures in Selected Genotypes of Groundnut in Relation to Late Leaf Spot Disease

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ABSTRACT

Six groundnut (*Arachis hypogaea* L.) cultivars were selected and categorized into resistant (Kadiri Harithandra), moderately resistant (ALR-3, JCG-8, GPBD-4) and susceptible (Narayani and K-6) based on disease severity using 1-9 scale. Morphological characters such as leaf area (mm^2), leaf thickness (µm), trichome density (per mm^2), stomatal frequency (per mm^2) and stomatal dimensions (µm) were estimated among the cultivars having different degrees of resistance. Leaf thickness and trichome density were more in resistant cultivar and less in susceptible cultivars. The stomatal frequency and size of stomata were less in resistant cultivar and more in susceptible cultivar.

Key words: *Groundnut, late leaf spot, Morphological resistance, Phaeoisariopsis personata*

Groundnut (*Arachis hypogaea* L.) is one of the important oil seed crops of India. Diseases of groundnut reduce yield and quality, and increase cost of production wherever the crop is grown. Among the groundnut diseases, late leaf spot (LLS) caused by *Phaeoisariopsis personata* (Berk. & Curt.) is the most serious fungal disease that accounts for more than 50% yield losses (Subrahmanyam *et al.,* 1982). 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. 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.

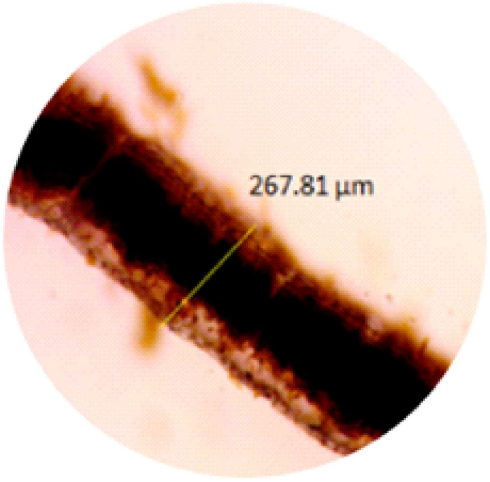
MATERIAL AND METHODS

Six groundnut genotypes obtained from Argricultural Research Station, Khadiri, Andhra Pradesh, i.e., Kadiri Harithandra (resistant to LLS), ALR3, JCG-8, GPBD-4 (moderately susceptible to LLS), Narayani and K-6 (susceptible to LLS) were selected to study the histological mechanisms of resistance in groundnut against late leaf spot disease. These genotypes were raised in 15 cm diameter cement pots with 50% sand and 50% soil with compost in a green house at the rate of three plants per pot. Four replications per each genotype were maintained. For the purpose of leaf anatomical studies, third fully expanded leaf from apex of one month old greenhouse grown plants from each genotype was cut, and utilized for the purpose. Structural defense parameters *viz.,* leaf area, leaf thickness, trichome density, stomatal frequency and stomatal dimensions were studied by following procedures described below.

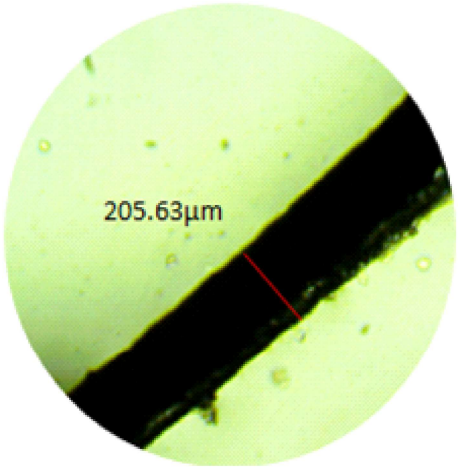
Area of quadrifoliate leaf (mm^2) of groundnut was calculated by using leaf area meter. Leaf samples of individual genotype were collected from 30 day old plants and thin sections were made using sharp stainless blade, placed on glass slide, covered with coverslip and leaf thickness was measured under LabdoMed LX300 microscope (150 X) with the help of calibrated ocular micrometer.

The samples for leaf surface studies were fixed and cleared in fixative containing glacial acetic acid and ethanol in 3:1 (v/v) ratio, and observed under the compound microscope. The diameter of the microscopic field was measured with the help of stage micrometer and the area of the field was caluculated. Number of trichomes counted in four microscopic fields under magnification of 20X randomly for each genotype and average was worked out and represented as number of trichomes per mm^2 (Tridevi, 2014).

Four healthy leaves were sampled from each genotypes at random ensuring one leaf from each replication. A thin film of adhesive (Quick fix) was applied. After drying, the film was removed randomly in between the veins and observed it under the microscope. The diameter of the microscopic field was measured with the help of stage micrometer and the area of the field was caluculated. The number of stomata were counted in four microscopic fields under magnification of 600X randomly, average was worked out and represented as number of stomata per $mm^2$and stomatal dimensions (length and width) were measured under LabdoMed microscope (600 X) with the help of calibrated ocular micrometer (Varadarajan and Wilson, 1973).



Resistant (Kadiri Harithandhra)



Susceptible (K-6)

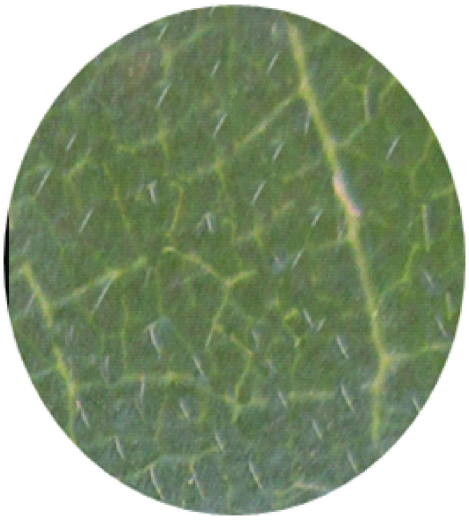
Plate 1. **Variation in leaf thickness in groundnut genotypes in relation to late leaf spot disease**

Abaxial surface

Resistant (Kadiri Harithandra)

Susceptible (K-6)

Adaxial surface



Resistant (Kadiri Harithandhra)

Susceptible (K-6)

Plate 2. **Trichome density in groundnut genotypes in relation to late leaf spot disease (per microscopic field 20X)**

Table 1. Assessment of morphological characters in selected groundnut genotypes in relation to late leaf spot disease

S. No.	Genotypes	Disease Reaction	Av. Leaf Area (mm^2)	Leaf Thickness (µm)	No. of Trichomes per mm^2		No. of Stomata per mm^2		Length of stomata (µm)		Width of stomata (µm)	
					Adaxial	Abaxial	Abaxial	Adaxial	Abaxail	Adaxial	Abaxial	Adaxial
1	Kadiri Harithandra	R	3155	271.55	44.12	170.59	103.13	106.21	22.95	20.43	11.92	10.22
2	ALR-3	MR	3202.5	252.62	17.65	111.76	118.21	112.01	26.33	24.65	13.63	12.75
3	JCG-8	MR	2467.5	239.97	17.65	123.53	126.01	109.41	28.05	21.25	15.32	11.93
4	GPBD-4	MR	2820	233.64	20.59	129.41	126.22	121.00	22.95	22.08	14.45	13.64
5	Narayani	S	3362.5	227.34	11.76	61.76	168.11	135.21	27.22	25.52	15.32	15.32
6	K-6	S	3292.5	208.40	23.53	91.18	174.00	129.00	28.05	23.83	16.15	15.33
SEm(±)			148.32	9.99	0.86	7.05	6.70	6.07	1.25	0.94	0.47	0.55
CD(P? 0.05)			444.08	29.87	2.57	21.11	20.04	18.16	3.76	2.81	1.42	1.64
CV(%)			9.73	8.36	7.54	11.58	9.86	10.23	9.68	8.17	6.55	8.33

*means of four replications

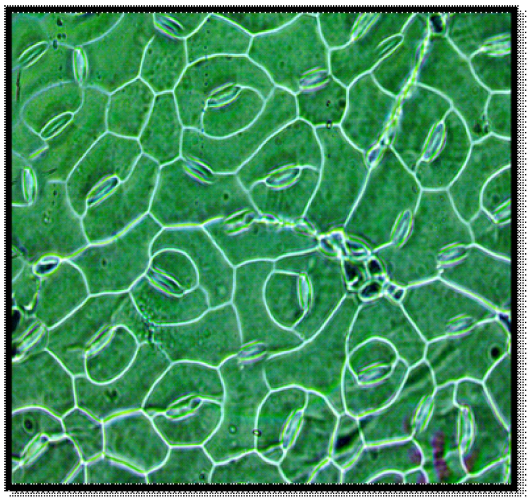
R= Resistant MR= Moderatly resistant S=Suceptible

Table 2. Correlation of late leaf spot disease with morphological characters of different groundnut genotypes

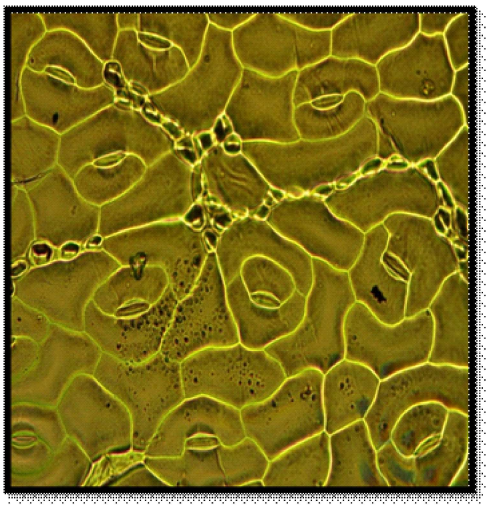
S. No.	Variable	Correlation co-efficient (r)
1	Leaf area (mm2)	0.492NS
2	Leaf thickness (µm)	-0.872*
3	Stomata on abaxial surface (mm2)	0.986**
4	Stomata adaxial surface (mm2)	0.897*
5	Trichomes on adaxial (mm2)	-0.585NS
6	Trichomes on abaxial surface (mm2)	-0.921**
7	Length of stomata on abaxial surface (µm)	0.709NS
8	Length of stomata adaxial surface (µm)	0.769NS
9	Width of stomata on abaxial surface (µm)	0.819*
10	Width of stomata on adaxial surface (µm)	0.924**

**significant at 1% level N=6 r tab= 0.811(5%) & 0.917(1%)
* significant at 5% level NS= Non significant

Distribution of stomata on the abaxial surface

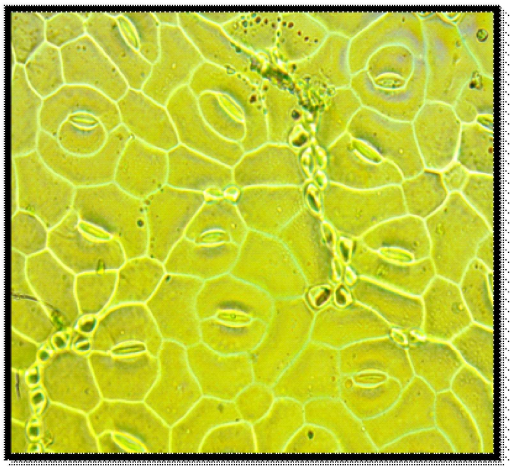


Susceptible (K-6)



Resistant (Kadiri Harithandra)

Distribution of stomata on the adaxial surface

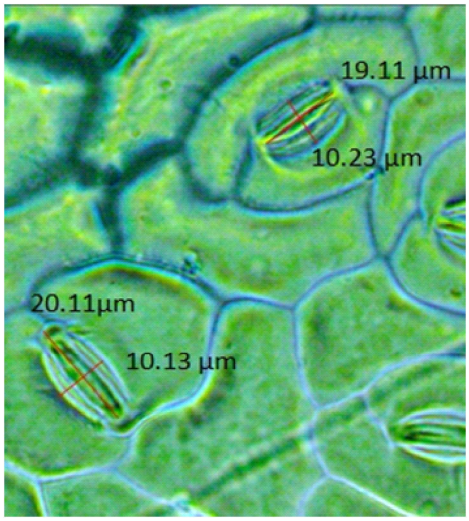


Susceptible (K-6)

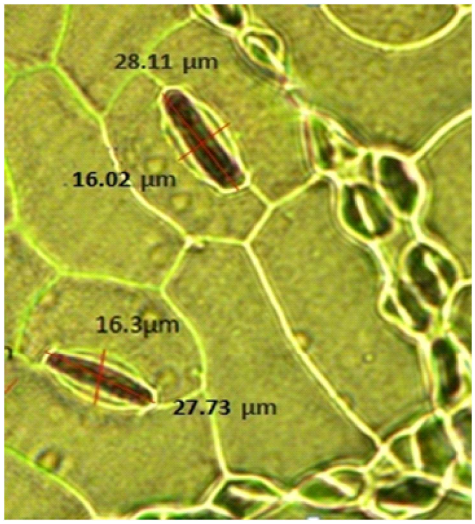
Resistant (Kadiri Harithandra)

Plate 3. Variation in stomatal frequency in groundnut genotypes in relation to late leaf spot disease (per microscopic field 600X)

Abaxial surface of leaf

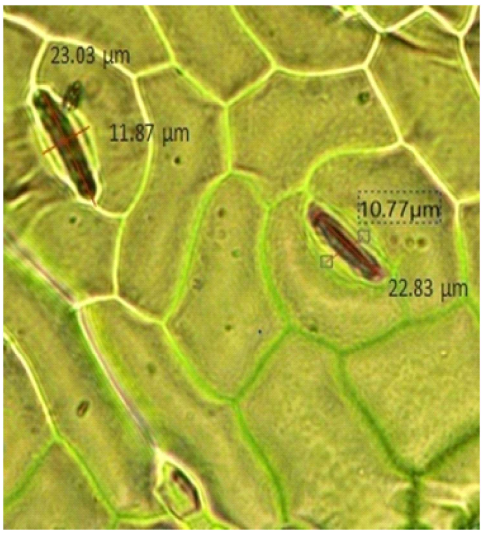


Resistant (Kadiri Harithandra)

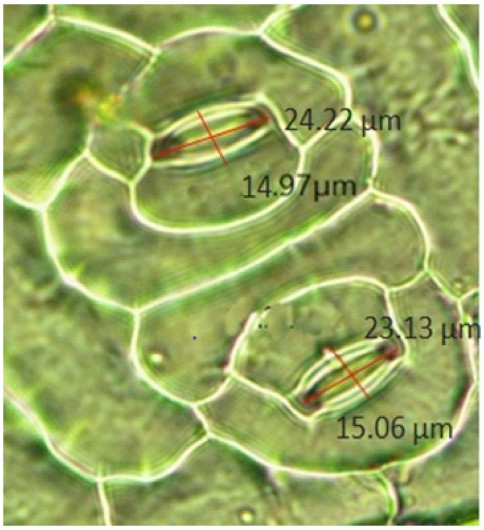


Susceptible (K-6)

Adaxial surface of leaf



Resistant (Kadiri Harithandra)



Susceptible (K-6)

Plate 4. **Stomatal aperture dimensions groundnut genotypes in relation to late leaf spot disease (600X)**

Disease severity was assessed on a 1-9 (Scale of Subrahmanyam *et al*., 1995). However the scale was modified by subtracting '1' as a common factor for all the numerical scale values in order to obtain 0 % PDI for no disease instead of 11.1 % PDI on 1-9 scale and PDI values were calculated based on the following formula.

$$PDI = \frac{[x_1(1\text{-}1)_+ x_2(2\text{-}1)_{+\ldots+} x_9(9\text{-}1)]}{N \times (9\text{-}1)} \times 100$$

Where, x is number of samples in each score and N is the total samples scored

RESULTS AND DISCUSSION

Among selected groundnut genotypes significantly highest leaf area was observed in susceptible genotype Narayani (3362.50 mm^2) and lowest leaf area was observed in moderately resistant genotype JCG-8 (2467.50 mm^2) (Table 1).

Significantly highest leaf thickness was observed in resistant genotype Kadiri Harithandra (271.55 µm) and significantly lowest leaf thickness was observed in the two susceptible genotypes K-6 (208.40 µm) and Narayani (227.34 µm). Significantly highest trichome density was noticed on abaxial surface of leaf compared to adaxial surface. On abaxial surface, number of trichomes were significantly high in resistant genotype Kadiri Harithandra (170.59 per mm^2) and lowest number of trichomes were observed in Narayani (61.76) (Table 1, Plate 1, 2).

More number of stomata was observed on abaxial surface compared to adaxial surface of leaf. On abaxial surface significantly highest stomatal frequency was observed in two susceptible genotypes K-6 (174 per mm^2) and Narayani (168.11 per mm^2), and the lowest number of stomata was recorded in resistant genotype Kadiri Harithandra (103.13 per mm^2) (Table 1, Plate 3).

Stomatal dimensions such as length and width of the stomata were high on abaxial surface compared to adaxial surface of leaf. On abaxial surface significantly highest stomatal length was recorded in two susceptible genotypes K-6 (28.05 µm) and Narayani (27.22 µm) and lowest stomatal length was recorded in resistant genotype Kadiri Harithandra (22.95 µm). Significantly highest stomatal width was recorded in two susceptible genotypes K-6 (16.15 µm) and Narayani (15.32 µm) and lowest stomatal width was recorded in resistant genotype Kadiri Harithandra (11.92 µm) (Table 1, Plate 4).

Among the selected morphological characters stomatal frenquency on adaxial (r = 0.897) and abaxial surface (r = 0.986) of leaf, stomatal dimensions *viz*., width of stomata on adaxial (r = 0.924) and abaxial surface (r= 0.819) of leaf were significant and positively correlated with PDI whereas leaf thickness (r = -0.872), trichome density on abaxial surface (r= -0.921) were significant and negatively correlated with PDI (Table 2).

Susceptible genotypes recorded less leaf thickness, less trichome density, higher frequency and size of stomata which may provide higher opportunity for penetration by the pathogens and thus resulted in high disease severity than that in resistant ones. These results are in agreement with studies conducted by different workers (Suryawanshi *et al*., 1994; Mayee and Suryawanshi, 1995 and Benagi, 1998) who 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.

CONCLUSION

It can be concluded 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. Tolerant genotype has higher leaf thickness and trichome density than the susceptible genotype (Trivedi 2014).

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