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Components of resistance to late leaf spot caused by *Phaeoisariopsis personata* in inter-specific derivatives of groundnut

S. PANDE[1]*, J. NARAYANA RAO[1] and S.L. DWIVEDI[2]

[1] *Natural Resource Management Program,*[2] *Genetic Resource Enhancement Program, International Crops Research Institute for the Semi Arid Tropics (ICRISAT), Patancheru, 502 324*

ABSTRACT: Five inter-specific derivatives of groundnut (*Arachis hypogaea*) along with two known susceptible cultivars were studied for components of resistance to *Phaeoisariopsis personata* in field and greenhouse. All the inter-specific derivatives showed significantly longer incubation and latent periods, lower lesion number, lesion frequency, smaller lesion diameter, lesser sporulation indices, less leaf area damage and less defoliation than susceptible cultivars in both environments. The correlation coefficients of incubation period, and latent period as LP_1 and LP_{50} were highly significant and positive but negative and highly significant with lesion number, lesion frequency, lesion diameter, sporulation index, leaf area damage, and defoliation for all the genotypes. Among these, latent period as LP_1, lesion diameter, sporulation index, and percentage defoliation were major components contributing to LLS resistance in groundnut.

Key words: *Arachis hypogaea*, components of resistance, inter-specific derivatives, *Phaeoisariopsis personata*, groundnut

Late leaf spot (LLS) of groundnut (*Arachis hypogaea L.*), caused by *Phaeoisariopsis personata* [(Berk. and Curt.) V. Arx]= *Cercosporidium personatum* (Berk. and Curt.) Deighton, is an economically important disease whereever groundnut is grown. It causes severe defoliation and reduces pod yields by more than 50% if the crop is not protected with chemicals (Smith and Littrell, 1980). Use of resistant cultivars is the best way to control these diseases but high levels of resistance are not available in cultivated groundnuts (Porter *et al.*, 1982). Several workers have reported partial resistance in cultivated groundnuts (Watson, 1987). This partial resistance can reduce the severity of LLS disease to some extent. The resistance includes several components that contribute to the reduction in the rate of disease development (Parlevliet, 1979; Subrahmanyam *et al.*, 1985). The objectives of this study were 1) to quantify the components of resistance to LLS in the field and greenhouse and 2) to determine correlations among resistance components in the field and in the greenhouse in inter-specific groundnut genotypes.

*Corresponding author: (Email: S. Pande@cgiar.org)

MATERIALS AND METHODS

Five inter-specific groundnut genotypes and two susceptible cultivars, having different levels of resistance to LLS were evaluated for quantification of different components of resistance in the field and in greenhouse environments during the 1997 and 1998 rainy seasons. These inter-specific genotypes were derived from crosses of cultivated groundnuts with wild *Arachis* species. The genotypes used in these experiments were ICGV 86699, (CS 29/1-B2-B1); ICGV 94108, [(CS 39 × CS 13) × (F 334A-B-14 × NCAC 2214)]; ICGV 94118, [(J 11 × CS 52) × (ICGS 44 × TEG 2)]; ICGV 96283, [(CS 9 × ICGS 44) × ICG 770]; ICGV 96284, [(CS 9 × ICGS 44) × ICG 7707]; Robut 33-1, (ICG 799); and TMV 2 (ICG 221). The wild *Arachis* parents involved in the crosses of these

inter-specific derivatives were, CS 9 = *A. hypogaea* × *A. cardenasii*; CS 13 = *A. hypogaea* × *A. cardenasii*; CS 29/1 = *(A. batizocoi* × *A. duranensis)* × *A. hypogaea*; CS 39 = *A. hypogaea* × *A. cardenasii*; CS 52 = *A. hypogaea* × *A. cardenasii*.

Field environment

The experiment was conducted in an Alfisol field at Patancheru. The plantings were done in the last week of June in both years. The experiment was conducted in a randomized complete block design with three replications. Each plot consisted of four rows of 9 m length with 60 cm inter- and 10 cm intra-row spacing. Sunflower crop was planted at 4 m wide around the experimental plot to avoid probable aerial contamination by other foliar fungal pathogens. At 35 days after sowing (DAS) the whole crop was inoculated with conidial suspension of LLS pathogen containing 20,000 conidia/ml late in the evening. Before fungal inoculation, the whole crop was given a light overhead sprinkler irrigation for 30 min to create leaf wetness on the foliage throughout the night. Thereafter, overhead irrigation was given every day for 30 min in the late evening for 10 days to maintain alternate wet (night) and dry (day) periods to obtain maximum disease development (Butler *et al.*, 1994). Calixin (Tridemorph) @ 250 ml/ 500 l of water per ha, was sprayed at 10-day interval from 30 DAS till maturity of the crop to control rust.

The components of resistance studied were: i) incubation period, ii) latent period as first sporulating lesion, and 50% of primary lesions sporulated, iii) lesion number/leaf, iv) lesion frequency, v) lesion diameter, vi) sporulation index, vii) percentage leaf area damage, and viii) percentage defoliation. Data on all components of resistance were recorded at 10-day interval from 10 days after inoculation (DAI) until crop maturity. The maximum disease was recorded at 50 DAI for lesion number, lesion frequency, lesion diameter, sporulation index, and percentage leaf area damage, but defoliation was recorded at 70 DAI in all the genotypes. In the beginning of the disease assessment, 20 random plants were tagged in each plot to study incubation and latent periods. Incubation period was recorded by observing all the tagged plants in each plot. Latent periods were identified by observing all the tagged plants every day with the aid of 20x magnifying lens. Fifteen quadrifoliate leaves were collected from the middle canopy of non-tagged plants in each plot for lesion numbers, lesion diameter and sporulation index at each observation. Total number of lesions/leaf were counted from all the 15 leaves and lesion diameter was recorded from 10 randomly selected primary lesions from each plot. For sporulation index, the leaves with lesions were kept in the moist chamber, (Petri dish with moist filter paper) and incubated at 25 ± 1° C with 12 h light and 12 h dark for 72 h to enhance sporulation. The intensity of sporulation was rated for 10 randomly selected mature lesions using stereoscopic microscope on a 1 to 9 scale where 1 = no lesion (no sporulation); 2 = up to 10% of the lesion area covered with fascicles and conidia (sparse sporulation); 3 = 11 to 20% of the lesion area covered with fascicles and conidia (slight sporulation); 4 = 21 to 30% of the lesion area covered with fascicles and conidia (moderate sporulation); 5 = 31 to 40% of the lesion area covered with fascicles and conidia (fair sporulation); 6 = 41 to 50% of the lesion area covered with fascicles and conidia (moderately high sporulation); 7 = 51 to 60% of the lesion area covered with fascicles and conidia (high sporulation); 8 = 61 to 80% of the lesion area covered with fascicles and conidia (very high sporulation); 9 = 81 to 100% of the lesion area covered with fascicles and conidia (dense sporulation). In the beginning of the disease assessment, five plants were selected at random from 20 tagged plants in each plot and all the leaves on the main stem of each plant were assessed for leaf area damage and defoliation. The leaf area damage by LLS was assessed by comparing each leaf with diagrams depicting leaves with known percentages of their areas affected (Hassan and Beute, 1977). The number of defoliated leaves on the main stem were counted at each assessment and percentage defoliation was calculated based on total and defoliated leaflets.

Greenhouse environment

The above seven genotypes were also used in greenhouse experiments for studying all the above components of resistance. Five seeds of each genotype were sown in 15 cm diameter plastic pots containing autoclaved Alfisol and farm

yard manure in 4:1 ratio (w/w). Two healthy plants were retained in each pot after germination and each pot served as a replication. The pots were arranged in a randomized complete block design with three replications. At 35 DAS both the plants in each pot were inoculated uniformly with the pathogen inoculum containing 20,000 conidia/ml with an atomizer in the evening. The inoculated plants were placed in a dew chamber at 23 ± 1° C during night and in the greenhouse during day to maintain wet and dry periods for 10 days (Butler *et al.*, 1994). The plants were then maintained in the greenhouse until the end of the experiment. The experiment was terminated at 50 DAI when all the tagged leaves of all the genotypes were completely defoliated. The minimum and maximum temperatures in the greenhouse during the period of the experiment were 19 to 24° C and 26 to 32° C in 1997 and 18 to 23° C and 19 to 24° C in 1998, respectively.

Data on all components of resistance were recorded at 5-day intervals from 10 DAI. Since in all the genotypes lesion number, lesion frequency, and percentage leaf area damage were maximum at 20 DAI and lesion diameter, sporulation index, and defoliation were maximum at 30 DAI, these data were used for analysis and presentation. In each pot, two healthy and fully expanded quadrifoliate leaves of the main stem of each plant were tagged for studying all the components of resistance. The lesion diameter and sporulation index were recorded from the non tagged leaves of both plants in each pot. The methodology of collecting the data on all the components of resistance in each pot in the greenhouse was similar to field study.

Data analysis was done separately for field and greenhouse by pooling the data of the year 1997 and 1998. The pooling of data was possible as the mean square error (MSE) and the mean values for both years were similar. Analysis of variance was carried out with PROC GLM (SASI, 1989).

RESULTS

Incubation period

Significant differences ($P<0.001$) were found in the incubation period between the environments and the genotypes but not among years (Table 1). The incubation period of all the genotypes was significantly shorter in the greenhouse than in the field. Among the inter-specific derivatives, the longest incubation period was recorded in ICGVs 86699, 96283, and 96284 in both environments whereas ICGVs 94108 and 94118 in field and ICGV 94118 in greenhouse showed significantly shorter incubation period.

Latent period

The latent period as first sporulating lesion

Table 1. Incubation period (IP), latent period in days from inoculation to first sporulating lesion (LP_1) and 50% lesions sporulated (LP_{50}) to late leaf spot disease for selected medium-maturing groundnut genotypes in field and greenhouse environments

	Days[a]					
	IP		LP_1		LP_{50}	
Genotype	Field	Greenhouse	Field	Greenhouse	Field	Greenhouse
ICGV 86699	16.7	15.2	25.0	24.8	34.2	32.2
ICGV 94108	14.2	13.2	19.8	17.8	25.5	22.2
ICGV 94118	14.2	12.0	20.2	18.0	26.2	22.7
ICGV 96283	16.8	15.0	25.5	25.2	34.2	31.8
ICGV 96284	16.7	15.2	25.8	25.5	34.0	32.3
Robut 33-1	10.7	8.0	16.8	16.0	20.0	19.8
TMV 2	9.8	10.0	14.3	14.5	17.5	17.5
CD 1%	1.48		2.33		1.99	

a = Mean of three replications; IP= Days for the appearance of the first symptom; LP_1= Latent period as first sporulating lesion; LP_{50}= Latent period as 50% lesions sporulated

(LP_1) was highly significant (P<0.001) between the genotypes but not between the environments and the years. But the latent period as 50% lesions sporulated (LP_{50}) were highly significant (P<0.001) between the environments and also between the genotypes but not among years. The LP_{50} of all the genotypes was significantly shorter in the greenhouse than in the field. Among the inter-specific derivatives, the longest latent period of LP_1 and LP_{50} were observed in ICGVs 86699, 96283, and 96284 in both the environments whereas ICGVs 94108 and 94118 showed shorter latent periods in both the environments (Table 1).

Lesion number

Highly significant differences (P<0.001) in the lesion number/leaf were found between environments and genotypes. The lesion number/leaf in the greenhouse were lower than in the field. All the inter-specific derivatives showed significantly lower lesion number/leaf than susceptible checks. Significantly fewer lesions occurred on ICGVs 86699, 96283, and 96284 in both environments (Table 2).

Lesion frequency

Lesion frequency was highly significant (P<0.001) between environments and genotypes. Significantly less lesion frequency was observed in all the inter-specific derivatives than both susceptible checks in both environments. The lowest lesion frequency was found in ICGV 86699 followed by ICGVs 96283, and 96284 in both environments (Table 2).

Lesion diameter

Highly significant differences (P< 0.001) were observed for lesion diameter between environments and genotypes. The lesion diameter was significantly larger in the greenhouse than the field. Among the inter-specific derivatives ICGVs 86699, 96283, and 96284 showed smallest lesion diameter than ICGVs 94108 and 94118 in both environments (Table 2).

Sporulation index

Sporulation index was found highly significant (P< 0.001) among genotypes but not between environments or years. All the inter-specific derivatives showed significantly (P<0.001) lower sporulation indices than either of the susceptible checks. Among the inter-specific derivatives ICGVs 86699, 96283, 96284 had lower soporulation indices than ICGVs 94108 and 94118 in both environments (Table 2).

Percentage leaf area damage

Differences among environments and genotypes were highly significant (P< 0.001) for percentage leaf area damage. The leaf area damage was greater in both susceptible cultivars

Table 2. Lesion number (LN), lesion frequency (LF), lesion diameter(LD), sporulation index(SI), percentage leaf area damage (LAD %) and percentage defoliation (DF %) to late leaf spot disease of selected groundnut genotypes in field and greenhouse environments

Genotype	LN[a]		LF[a]		LD[a]		SI[a]		LAD (%)[a]		DF(%)[a]	
	Field	Green house	Field	Green house	Field	Green house	Field	Green house	Field	Green house	Field	Green house
ICGV 86699	46.2	34.7	1.8	1.5	2.1	2.6	2.5	3.1	3.5	1.8	69.6	69.8
ICGV 94108	93.3	84.9	2.9	3.8	3.8	4.4	4.8	5.4	5.8	5.4	79.6	90.6
ICGV 94118	111.9	110.9	3.4	4.7	4.1	4.5	4.7	4.7	5.6	11.9	83.7	100.0
ICGV 96283	48.0	28.9	1.9	1.0	2.3	2.6	2.4	2.9	3.5	1.1	75.4	61.5
ICGV 96284	52.8	37.6	1.9	1.3	2.2	2.7	2.6	2.7	3.9	1.4	71.8	73.9
Robut 33-1	366.8	87.9	13.2	3.2	3.9	5.0	7.5	7.3	12.7	15.3	92.3	100.0
TMV 2	437.5	173.9	12.4	5.1	5.1	6.8	8.3	8.7	21.4	46.1	100.0	100.0
CD 1%	52.6		2.41		0.97		1.18		7.97		7.97	

a = Mean of three replications; LN= Number of lesion/leaf; LF= Lesion frequency; LD= Lesion diameter; SI= Sporulation index; LAD= Leaf area damage; DF= Defoliation

in the greenhouse environment than in the field, but it was greater in the field environment in all the inter-specific derivatives except ICGV 94118. Among the inter-specific derivatives ICGVs 86699, 96283, and 86284 showed less leaf area damage than the rest of the derivatives in both environments (Table 2).

Percentage defoliation

Highly significant differences ($P< 0.001$) were recorded between the genotypes. In general, the percentage defoliation was greater in greenhouse environment except in ICGV 96283 than in the field environment. The lowest percentage of defoliation among the inter-specific derivatives was recorded in ICGV 86699 followed by ICGVs 96283, and 96284 in both environments (Table 2).

Correlations among components of resistance

Correlation coefficients among components of resistance within each environment were highly significant [($P<0.001$) (Table 3)]. The incubation period (IP) and latent periods as LP_1 and LP_{50} were significantly correlated in both environments. Latent period as LP_1 was significantly ($P<0.001$) correlated with LP_{50} in field and greenhouse environments. The lesion number/leaf and lesion frequency were negatively and highly significantly correlated ($P<0.001$) with IP, LP_1, and LP_{50} in both environments. Correlations of lesion diameter was highly significant and negative ($P<0.001$) with IP, LP_1 and LP_{50} whereas positive and highly significant with lesion number and lesion frequency in both the environments. Sporulation index was correlated negatively and highly significantly ($P<0.001$) with IP, LP_1, and LP_{50} but correlated highly positively with lesion number, lesion frequency and lesion diameter in both the environments. Correlations of percentage leaf area damage with IP, LP_1, and LP_{50} were negative and highly significant ($P<0.001$) but positive and highly significant with lesion number, lesion frequency, lesion diameter and sporulation index in both environments. Similarly percentage defoliation was highly significant ($P<0.001$) and negatively correlated with IP, LP_1, and LP_{50} but positively correlated with high significance with lesion number, lesion frequency,

Table 3. Correlation coefficients among components of resistance of selected groundnut genotypes to late leaf spot disease in field and greenhouse

		IP	LP_1	LP_{50}	LN	LF	LD	SI	LAD	DF
IP	F		0.957	0.968	-0.912	-0.882	-0.821	-0.905	-0.718	-0.838
	GH		0.878	0.878	-0.737	-0.622	-0.873	-0.872	-0.776	-0.645
LP_1	F			0.968	-0.842	-0.807	-0.872	-0.859	-0.657	-0.835
	GH			0.985	-0.805	-0.811	-0.932	-0.888	-0.726	-0.711
LP_{50}	F				-0.883	-0.843	-0.861	-0.930	-0.731	-0.867
	GH				-0.809	-0.805	-0.940	-0.885	-0.725	-0.743
LN	F					-0.951	0.660	0.922	0.782	0.832
	GH					-0.859	0.829	0.702	0.835	0.715
LF	F						0.646	0.872	0.637	0.791
	GH						0.756	0.642	0.646	0.719
LD	F							0.726	0.506	0.745
	GH							0.924	0.823	0.651
SI	F								0.808	0.824
	GH								0.769	0.487
LAD	F									0.672
	GH									0.505

F = Field environment; GH = Greenhouse environment; IP = Incubation period; LP_1 = Latent period as first sporulating lesion; LP_{50} = Latent period as 50% lesions sporulated; LN = Lesion number; LF = Lesion frequency; LD = Lesion diameter ; SI = Sporulation index; LAD = Leaf area damage; DF=Defoliation

lesion diameter, sporulation index, and leaf area damage in both environments.

DISCUSSION

All the five inter-specific derivatives showed significantly longer incubation and latent periods, fewer lesions/leaf, smaller lesion diameters, lower sporulation indices, less leaf area damage and less defoliation than susceptible TMV 2. Though ICGVs 94108 and 94118 showed shorter incubation period than the rest of the inter-specific derivatives, but they had longer incubation period than the susceptible cultivars. Several workers (Waliyar *et al.*, 1993) have reported longer latent periods, smaller lesion size, lower sporulation index, less leaf area damage and lower percentage of defoliation in resistant groundnut cultivars.

The shorter incubation and latent periods in the greenhouse in all the genotypes were probably because of the favourable conditions created in the greenhouse after inoculation for the development of LLS. Similar results of low incubation and latent periods in the greenhouse environment than field were also recorded by Watson (1987). The correlation coefficients of incubation period with both the latent periods were highly positive and in agreement with the results obtained by Chiteka *et al.* (1988b). The lowest number of lesions/leaf were observed in ICGVs 86699, 96283, and 96284 than the rest of the genotypes. In the present study the number of lesions/leaf were found more in the field environment than the greenhouse environment. This may be because of the continuous deposition of the conidia on the foliage during the crop season. In contrast to the field, in the greenhouse only one primary infection period was used for total lesion number and no secondary cycles occurred. These results are in agreement with those reported by Walls *et al.*, (1985). Waliyar *et al.*, (1993), observed the inconsistency in the lesion number and reported that it was not a reliable component for selecting resistance. Contrary to these results, the lesion number in the present study was found consistent in both years and in both environments in all the genotypes.

The longer the incubation period, the lower the lesion number and hence lesion number was highly negatively correlated with incubation period and latent period as LP_1, and LP_{50}. Similar trends were observed for lesion frequency in both environments and in both years. Significant differences in lesion diameter were observed among genotypes and also in environments (Watson, 1987). In the present study, the lesion diameter was larger in the greenhouse than in the field environment. Contrary to this observation, Chiteka *et al.* (1988a) found larger lesion diameter in the field than in the greenhouse. Lesion diameter was correlated negatively and significantly with IP, LP_1, and LP_{50}. Similar results were obtained by Chiteka *et al.*, (1988b). Lesion diameter was significantly and positively correlated with lesion number and lesion frequency. The lesion diameter seems to be an important component for selecting resistant genotypes.

Differences in sporulation index were found among genotypes as also reported by Watson (1987). The mean sporulation index was highest in all the genotypes in greenhouse than in field. These results are in contrary to the results obtained by Chiteka *et al.*, (1988a). High sporulation index in the greenhouse is usually expected because of the congenial conditions for the LLS development. In general, the genotypes that retard sporulation of the pathogen also retard its infection rate. The reduced sporulation plays an important role in reducing the rate of disease increase (Gobina *et al.*, 1983). Parlevliet (1979) also reported that the rate of apparent infection was affected proportionately with reduced or slowed down sporulation. Sporulation index showed a highly significant negative correlation with IP, LP_1, and LP_{50}. These results are in agreement with the results obtained by Chiteka *et al.* (1988b). Sporulation index also showed highly positive and significant correlation with lesion number, lesion frequency, and lesion diameter. Differences in percent leaf area damage were found among the genotypes and also between the environments as reported by Subrahmanyam *et al.*, (1985). The present study indicated that the components of resistance in field, and greenhouse showed similar trends in all the genotypes. Hence screening in either of the environments would be useful in identifying resistant lines.

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