Components of Resistance to an Indian Source of *Cercospora Arachidicola* in Selected Peanut Lines[1]

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ABSTRACT

Cercospora arachidicola Hori is one of the most important foliar pathogens worldwide that limits peanut production in farmers' fields. Earlier screening trials allowed us to identify lines with field resistance to early leafspot. In order to determine the components of resistance of these lines and other lines reported to be resistant elsewhere, 19 peanut genotypes (*Arachis hypogaea* L.) were evaluated by the detached leaf technique using an isolate of *Cercospora arachidicola* from the ICRISAT Center in India. Significant differences were observed among genotypes for all components of resistance included in the study. With a few exceptions, early leafspot- resistant genotypes (ICG nos. 8298, 6902, 6284, 1703, 10900, 7878, 9989 and 10920) exhibited longer incubation periods, reduced sporulation, smaller lesion diameters and lower infection frequencies than susceptible lines. Genotypes ICG 8298 and ICG 6902 were the most resistant, while ICG nos. 221, 7827 and 6340 were the most susceptible to early leafspot. A few lines had resistant reactions to some components but susceptibility to others.

Key Words: *Arachis hypogaea*, early leafspot, *Cercospora arachidicola*, groundnut, peanut, disease resistance.

Early leafspot of peanut caused by *Cercospora arachidicola* Hori (CA). is an economically important foliar disease in most countries where peanuts (*Arachis hypogaea* L.) are grown. This disease reduces the green leaf area available for photosynthesis and stimulates leaflet abscission leading to extensive defoliation (10). Damage is more serious when the crop is attacked by both early and late (*Cercosporidium personatum* (Berk. & Curt.) Deighton) leafspot pathogens. Pod yield losses due to both pathogens together may range from 10 to 60% (4,10,18). Although effective chemical control methods are available in many areas of the world, their applications are limited because of high costs and the possible existence of fungicide-tolerant strains of the pathogens in developing countries (2,9). Consequently, development of disease resistant cultivars is a high priority in international programs.

Screening peanut germplasm for resistance to the early leafspot pathogen is in progress in several areas of the world and genotypes with resistance or tolerance have been identified (1,3,7,10,11). Successful screening for resistance can partially be attributed to the regular occurrence of epidemics in those regions. Many studies on the components of resistance to *C. arachidicola* have been conducted (3,6,8,12,13,14,), but most of this work concerns pathogen isolates from the USA. Screening trials for resistance to early leafspot in India (Pantnagar), Nepal and Malawi (Lilongwe), where early leafspot epidemics occur annually, have shown that several cultivars and breeding lines reported as resistant elsewhere had variable reactions in these locations (19,20).

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Foster *et al.* (3) reported varying levels of susceptibility in "resistant" lines in an experiment using spores collected in the USA. Although components of resistance to both early and late leafspots in India have been reported (12), none of the genotypes used in a detached leaf test by Nevill (12) were resistant to early leafspot. Studies of components of resistance in laboratory experiments were undertaken at ICRISAT, Patancheru, India using sources of resistance to early leafspot in genotypes that were previously selected in our field screening trials in India (19,20).

Materials and Methods

Nineteen peanut genotypes were selected for this study based on their reaction to *C. arachidicola* in India (18,19), USA (1,7,11,14) and Malawi (Waliyar unpublished data). Two local cultivars ICG 221 (TMV 2) and ICG 7827 (JL 24), known to be susceptible to early leafspot, were included in the test. Seed were sown in the glasshouse in a mixture of red sandy soil (Alfisol) and farmyard manure (4:1, V/V) in plastic pots (20 cm diameter). Three seeds were planted in each pot and plants were later reduced to two per pot. Day-time air temperatures in the glasshouse during the experiment ranged from 25 to 30 C. When plants were 30 days old, quadrifoliate leaves from either the second or third fully expanded leaf on the main stem were excised through the pulvinus and arranged in a randomized block design in plastic trays containing sterile river sand (17). Each genotype was replicated 12 times and each replicate consisted of one leaf having four leaflets. The test was repeated three times, but only results from one test are reported in this paper .

A cyclone spore collector was used to harvest conidia from sporulating lesions on detached leaves of the very susceptible cultivar TMV2, which was inoculated with an isolate of *C. arachidicola* collected from naturally infected leaf lesions at ICRISAT Center, India. Leaves were inoculated at room temperature and incubated in a growth chamber at 25 C with relative humidity of about 95% and with a 12 hr photoperiod. Inoculum was prepared by suspending conidia in sterile distilled water containing the surfactant Tween 80 (polyoxyethylene sorbitan monoleate) at 10 drops L^{-1}. The suspension was adjusted to a concentration of 50,000 conidia ml^{-1} and applied to leaves with an atomizer.

Number of lesions on inoculated leaves was recorded at daily intervals beginning 7 days after inoculation. When the number of lesions ceased to increase, total leaf area was measured with a leaf area meter (Hayashi Denkoh Co. Ltd., Tokyo, Japan). Necrotic area of leaflets (i.e. lesions were estimated using a Delta-T Area Meter (Delta-T Devices Ltd., Cambridge, England) equipped with a narrow band filter. Lesion diameter was estimated by measuring the diameter of 10 randomly selected lesions. Intensity of sporulation was estimated using a 1-5 scale (where 1= no sporulation and 5= profuse sporulation) under a stereoscopic microscope.

The following variables were calculated: incubation period (IP = number of days to appearance of 50% of the total number of lesions); infection frequency (IF = number of lesions per cm^2 leaf area); percentage necrotic leaf area; and leafspot reaction index (the product of percent necrotic leaf area and sporulation intensity).

Angular transformation was used where necessary to achieve normality of data and homogeneity of error variance. Analysis of variance was performed on all components of resistance including: incubation period, infection frequency, intensity of sporulation, leafspot reaction index, total lesion area, lesion diameter, and leaf area damage by early leafspot, compared to susceptible checks. Spearman rank correlation coefficients were computed between all possible pairs of variables to detect significant relationships between components of resistance (6) as well as with field scores (estimated on a 1-9 scale), obtained during the 1987 rainy season at ICRISAT Center, India (19,20). Genotypes were ranked for selected components in order of increasing resistance. Duncan's multiple range test was used to detect significant differences between means.

Results

Disease symptoms began appearing on leaflets of susceptible cultivars within 7 days after inoculation (Table 1). Significant ($P<0.001$) genotype differences were found for all resistance components. Average incubation period ranged from 11.6 days in ICG 6340 to 15.6 days in ICG 8298. The genotype ICG 8298 had the lowest infection frequency (1.73 lesion cm^{-2}), smallest lesions (average diameter of 0.7mm at 15 days and 1.2 mm at 20 days after inoculation (DAI)) and the longest incubation period. A breeding line with an overall susceptible reaction, ICGS 11, had a very small percent leaf area damage at 15 days after inoculation (0.83%) and 2.04% at 20 days after inoculation (Table 1). Depending on the components of resistance rated, genotypes ICG nos 8298, 6902, 7878, 7885, 9989, 1703, 6284, 10900, 10920, 9294, and 8339 were classified as resistant. The following lines were susceptible to most of components measured: ICG nos 221, 7827, 6340, 10940, 6330, 2711, 1710, and ICGS 11. The genotype ICG 7878 had a short incubation period (12.9 days), but the lowest intensity of sporulation (1.70 on a 1-5 scale). The entry ICG 10900 had a relatively high intensity of sporulation (2.38 on a 1-5 scale), but a longer incubation period (14.8 days), low infection frequency (2.83 lesion cm^{-2}) and leaf area damage (1.37% at 20 DAI), compared to the susceptible genotype, ICG 6340, which had 3.07(on a 1-5 scale), 11.6 days, 3.97 lesions cm^{-2} and 2.79%, respectively, for the same components. Another line, ICG 10920 had a resistant reaction to some components but susceptibility to others. Infection frequency for ICG 10920 was the lowest (1.69 lesion cm^{-2}), its incubation period was long (15.3 days) and its percent leaf area damage was low (0.73%) but its sporulation intensity (3.50 on 1-5 scale) was high and it had a large lesion diameter (2.22 mm at 20 DAI) (Table 1).

Genotype rankings for selected components of resistance are presented in Table 2. Resistant genotypes, ICG nos 8298, 6902, 6284, 1703, 10900, 7878, 9989, and 10920 consistently occur in the bottom half of the rankings. Eight susceptible genotypes, ICG nos 221, 7827, 6340, 10940, 6330, 2711, 1710, and ICGS 11 were found in the top half of the rankings. The three genotypes ICG nos 221, 7827 and 6340 were determined to be the most susceptible; ICG 8298 and ICG 6902 were believed to be the most resistant, and consistently rank among the five best genotypes for all resistance components (Table 2). Three other genotypes, ICG nos 7885, 8339 and 9294, had moderate resistance and varied in their rankings depending on the component of resistance measured. Genotype rankings for incubation period were not correlated with other components of resistance (Table 3). Genotype rankings for field score rankings were significantly correlated with all resistance components except incubation period. Angular transformations made little difference in the results and are therefore not included in the table.

Discussion

Peanut genotypes showed significant differences ($P<0.001$) for all components of resistance measured in the laboratory using the detached leaf technique. With a few exceptions, genotypes classified as resistant to CA exhibited longer incubation periods, lower intensities of sporulation, and smaller lesion size than susceptible genotypes. This is in accordance with the hypothesis of partial resistance proposed by Parlevliet (13) who stated that several components of resistance contribute to the reduction in the rate of epidemic progress. Savary and Zadoks (15) reported that for peanut rust disease, infection efficiency, latent period duration and sporulation were significantly correlated with the area under the disease progress curve. Exceptions to this pattern,

Table 1. Components of resistance to *Cercospora arachidicola* in 19 peanut genotypes in detached leaf studies*.

ICG No.	Identity	IP	IF	SP	TLA (%)	LSRI	LD (mm) 15 D	LD (mm) 20 D	LAD (%) 15 D	LAD (%) 20D
6340	PI 350680	11.6e	3.97ab	3.07c-e	9.22c-e	28.40c-f	1.27cd	2.07b-e	1.43a	2.79ab
221	TMV 2	11.8e	3.76ab	4.33a	12.80bc	52.39ab	1.81a	2.73a	1.44a	2.83ab
6330	PI 270806	11.9de	4.98a	2.72d-g	10.03b-e	29.19d-g	1.38bc	2.01b-e	1.54a	3.02ab
2711	NC 5	12.3de	3.89ab	2.57e-g	10.38b-d	28.51e-g	1.22c-e	2.00b-e	1.31a	2.43bc
7827	JL 24	12.8c-e	3.78ab	4.33a	14.29ab	63.54a	1.64ab	2.98a	1.30a	3.08ab
10940	PI 476176	12.8c-e	3.19bc	2.96d-f	17.82a	51.57ab	1.36bc	2.33b	1.56a	3.16a
7878	NC Ac 10811A	12.9c-e	2.85b-d	1.70i	12.43bc	19.98e-g	0.98d-h	1.53f-i	0.63c	1.50d-f
-	ICGS 11	12.9c-e	3.71ab	3.85ab	10.96b-d	42.85bc	1.30cd	2.29bc	0.83bc	2.04cd
9294	58-295	13.0c-e	2.72b-d	2.90d-f	9.50c-e	25.41d-g	1.23c-e	1.84d-h	1.22ab	2.46bc
1710	NCAc 17135	13.1c-e	3.13bc	2.94d-f	10.65b-d	30.50d-f	1.14c-f	1.93c-f	1.19ab	2.52a-c
6284	NC Ac 17500	13.2b-e	2.61b-d	2.27gh	7.13de	15.20gh	0.90e-h	1.39i	0.63c	1.22ef
1703	NC Ac 17127	13.5bc	1.84cd	2.50fg	7.64de	20.16e-g	0.98d-h	1.45h-i	0.65c	1.19ef
9989	US 403 Red	13.5b-d	3.84ab	1.79hi	10.13b-e	19.61e-g	0.82f-h	1.55f-i	0.75c	1.40d-f
7885	PI 381622	14.0a-c	2.74b-d	2.94d-f	8.88c-e	24.14c-f	1.09c-g	1.81e-h	0.78c	1.76de
8339	NC Ac 18091	14.2a-c	3.04b-d	3.14cd	9.86c-e	33.40c-e	1.10c-g	1.88d-g	0.67c	1.39d-f
10900	PI 476033	14.8ab	2.83b-d	2.38g	8.82c-e	23.43d-g	0.99d-h	1.57f-i	0.76c	1.37d-f
6902	NC Ac 17894	15.2a	2.21cd	2.27gh	6.45de	15.00fg	0.80gh	1.48g-i	0.52c	1.03f
10920	PI 476152	15.3a	1.69d	3.50bc	10.41b-d	37.27b-d	1.13c-f	2.22b-d	0.73c	1.38d-f
8298	NC Ac 18045	15.6a	1.73d	1.81hi	5.75e	11.04g	0.69h	1.18i	0.39c	0.80f
SE±		0.51	0.41	0.15	4.45	1.29	0.10	0.12	0.13	0.22
CV%		13.1	46.6	18.6	49.8	43.9	29.9	22.6	47.9	38.0
DF		189	198	162	155	186	198	198	198	198

* Mean of 12 replications; means followed by same letters are not significantly different at = 0.05 level according to Duncan's Multiple Range Test. IP = Incubation period (days); IF = Infection frequency (lesion cm^{-2}); SP = Intensity of sporulation; TLA=Total lesion area; LSRI = Leaf spot reaction index (TLA % x SP); LD = Lesion diameter; LAD = Leaf area damage by early leaf spot.

however, were observed - e.g. ICG 7878 had the lowest intensity of sporulation, but its incubation period was short (12.9 days).

Ranking genotypes for number of lesions was inconsistent across repeated tests and does not form a reliable method of detecting the resistant genotypes using the detached leaf technique(12). Although the effect of resistance on lesion number is difficult to measure due to inconsistencies from one experiment to another, it may still be important for disease progress. Similarly, infection frequency and percent leaf area damaged were correlated (as expected), but were inconsistent across repeated tests (Waliyar *et al.*, unpublished data).

Significant differences ($P<0.001$) among genotypes were observed in these tests for sporulation intensity. Genotypic differences ($P<0.001$) were also found for necrotic leaf area.

Table 2. Ranking of 19 peanut genotypes for incubation period (IP), intensity of sporulation (SP), infection frequency (IF), lesion diameter (LD) at 20 DAI, leaf area damaged (LAD) at 20 DAI.

ICG No	IP (days)	ICG No	SP	ICG No	IF lesion cm^{-2}	ICG No	LD20 (mm)	ICG No	LAD (%)
6340	11.6	7827	4.3	6330	5.0	7827	3.0	10940	3.2
221	11.8	221	4.3	6340	4.0	221	2.7	7827	3.1
6330	11.9	ICGS11	3.8	2711	3.9	ICGS11	2.3	6330	3.0
2711	12.3	10920	3.5	221	3.8	10940	2.3	6340	2.8
10940	12.8	6340	3.1	7827	3.8	10920	2.2	221	2.8
7878	12.8	8339	3.1	9989	3.8	6340	2.1	1710	2.5
7827	12.9	7885	3.0	ICGS11	3.7	2711	2.0	9294	2.5
9294	13.0	10940	3.0	10940	3.2	6330	2.0	2711	2.4
ICGS11	13.1	1710	2.9	1710	3.1	1710	1.9	ICGS11	2.0
1710	13.1	9294	2.9	8339	3.0	8339	1.9	7885	1.8
6284	13.2	6330	2.7	7878	2.9	7885	1.8	7878	1.5
1703	13.5	2711	2.6	10900	2.8	9294	1.8	8339	1.4
9989	13.5	1703	2.5	7885	2.7	9989	1.6	9989	1.4
7885	14.0	10900	2.4	9294	2.7	10900	1.6	10900	1.4
8339	14.2	6284	2.3	6284	2.6	1703	1.5	10920	1.4
10900	14.8	6902	2.3	6902	2.2	6902	1.5	1703	1.2
6902	15.0	8298	1.8	1703	1.8	7878	1.5	6284	1.2
10920	15.1	9989	1.8	8298	1.7	6284	1.4	6902	1.0
8298	15.7	7878	1.7	10920	1.7	8298	1.2	8298	0.8
SE±	0.51		0.15		0.41		0.12		0.22
CV(%)	13.1		18.6		46.6		22.6		38.0
DF	198 (9)*		162(26)		198		198		198

* Number of missing values

Table 3. Spearman rank correlation coefficients among components of resistance in 19 peanut entries to *C. arachidicola* measured in a detached leaf test.

	IP*	IF	SP	LSRI	TLA	LD15	LD20	LAD15	LAD20	FS
IP	1.00	-0.15	0.24	-0.11	-0.20	0.18	0.21	0.19	0.02	0.39
IF		1.00	0.64	0.60	0.67	0.70	0.73	0.74	0.82	0.63
SP			1.00	0.82	0.61	0.86	0.93	0.78	0.83	0.88
LSRI				1.00	0.85	0.53	0.71	0.46	0.67	0.57
TLA					1.00	0.50	0.65	0.46	0.68	0.47
LD15						1.00	0.95	0.97	0.92	0.92
LD20							1.00	0.91	0.91	0.92
LAD15								1.00	0.93	0.88
LAD20									1.00	0.85
FS										1.00

*IP = Incubation period (days); IF = Infection frequency; SP = Intensity of sporulation; LSRI = Leaf spot reaction index (TLA% x SP); TLA = Total lesion area; LD = Lesion diameter at 15 or 20 days after inoculation (DAI); LAD = Leaf area damage by early leaf spot at 15 or 20 DAI; FS = Field score on a 1-9 scale.

However, Spearman rank correlation coefficients for intensity of sporulation and percent necrotic leaf area were not significant. Therefore, differences in intensity of sporulation are concluded, herein, to be due to genuine genotypic influence rather than differences in necrotic leaf area. As previously reported (5,6), the intensity of sporulation is a reliable disease component for detecting resistant genotypes. Given the relatively high importance of this component to initiation and development of epidemics, the range of sporulation intensity across genotypes (from 1.7 to 4.3 on a 1-5 scale) is sufficient to differentiate between resistant and susceptible genotypes. Because the intensity of sporulation was reduced by 2.5 times in resistant genotypes as compared to susceptible ones, this could result in equivalent reduction in inoculum levels and lead to a substantial reduction in epidemic progress.

Variations observed in lesion diameter (1.2 to 3.0 mm) are perhaps more important when translated to an expression of lesion area (1.1 to 7.1 mm^2). Necrotic leaf area damaged reflects and the potential area available for inoculum production. Our results indicated that lesion diameter was consistent across repeated tests and correlated significantly with all other components of resistance except incubation period. Because lesion area has a direct influence on sporulation, and because there were significant genotypic differences for lesion size, this component has importance when testing for early leafspot resistance. It is noteworthy that Ricker *et al.* (14) did not find significant differences in lesion diameter estimated on 20 peanut genotypes during their investigations.

Although significant differences among genotypes were observed for percent defoliation and time of leaflet loss, consistency of results was lacking in detached leaf tests (Waliyar *et al.*, unpublished data). Because of this inconsistency, we did not include leaflet defoliation results in this report. Nevertheless, percent defoliation in the plant is too important to be neglected in resistance breeding because it affects the remaining green leaf area available for photosynthesis. For a reliable conclusion as to its relative importance, this component should be studied in the field rather than with detached leaf techniques.

Lines PI 350680 (ICG 6340) and NC 5 (ICG 2711) have been reported to be resistant to early leafspot in the USA (8,11,14). Laboratory studies of components of resistance showed that PI 350680 was susceptible and NC 5 was moderately susceptible, when using a CA isolate from India. Variable reactions of lines may be due to variation in pathogen virulence or, possibly, physiological races in different locations. In the case of late leafspot (CP), however, Shew *et al.* (16) reported stable resistance to CP when comparing CP isolates from the USA and Thailand. In the case of early leafspot, the breeding strategies should take into consideration potential virulence factors of the pathogen and geographical (climatic) environments.

Because components of peanut leafspot interaction were not fully complimentary, the utilization of resistance sources in individual breeding programs requires detailed study. It should be possible, however, to develop a breeding strategy in which diverse types of disease reactions are combined to enhance the levels of resistance in the cultivated species.

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