Components of Resistance to *Puccinia arachidis* in Peanuts

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ABSTRACT

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Thirty peanut genotypes were inoculated with uredospores of the rust fungus *Puccinia arachidis* in a replicated glasshouse trial, and components of disease resistance—incubation period, infection frequency, pustule diameter, percent ruptured pustules, and percent leaf area damaged—were studied for a single cycle of infection. All components studied were significantly correlated with one another and with mean field rust scores taken over several seasons. Incubation period was negatively correlated with the other components, which were positively correlated with one another. Resistant and susceptible genotypes were readily separated on the basis of the resistance components measured in the glasshouse trial, but classification of moderately resistant genotypes was less effective by this method than by use of field scores. A glasshouse screening method could be useful in areas where rust epidemics do not occur or are irregular in occurrence or where other foliar diseases interfere with field screening. The measurement of epidemiologically significant characters will allow the identification of rate-limiting resistance, which is likely to be more stable than immunity.

Additional key words: groundnut, screening methods, slow rusting.

Rust of peanut (*Arachis hypogaea* L.) caused by *Puccinia arachidis* Speg. has increased in importance in recent years. Before 1969, the disease was largely confined to South America and the Caribbean, with occasional outbreaks in the southernmost peanut-producing areas of the United States. Since 1969, rust has spread to almost all peanut-producing areas of the world (1,7,21). Yield losses from rust are substantial, damage being particularly severe if the crop is also attacked by the two major leafspot fungi, *Cercospora arachidicola* Hori and *Cercosporidium personatum* (Berk. & Curt.) Deighton (23). Although the foliar diseases can be controlled by certain fungicides, these fungicides are becoming more costly and may not be readily available to the small-scale farmer of the semiarid tropics (6). Therefore, considerable research in recent years has concerned the exploitation of genetic resistance (2,3,7,20).

At the ICRISAT Center, which is situated near Hyderabad, India, 10,000 peanut germ plasm lines, collected from many countries and maintained in the Genetic Resources Unit, were screened in the field for resistance to rust during the years 1977–1982. Previous reports of resistance were confirmed, and several new sources were identified (22,23). In the Hyderabad area, rust develops early in the rainy season (June to October) on susceptible genotypes and causes severe damage to the foliage, with resulting large yield losses. On more resistant genotypes, the disease appears later, builds up only slowly, does little apparent damage to the foliage, and causes only small losses in yield (23). On susceptible genotypes, numerous large elevated uredosori develop on the lower surface of the leaf, rupture, and sporulate profusely. Colonies of secondary uredosori later develop around the original uredosori, and the leaflets turn yellow and wither. On resistant genotypes, the uredosori are fewer in number, slightly depressed, small, and may not rupture to release the comparatively few spores produced. The affected leaflets show only limited necrosis (22).

The regular severe epidemics of rust that occur on peanuts grown during the rainy season at the ICRISAT Center facilitate field screening for resistance to the disease. However, this may not be feasible in some parts of the world where rust occurs sporadically. An alternative approach is to screen genotypes in the glasshouse under controlled conditions and with artificial inoculation. This article describes investigations on the components of rust resistance undertaken to obtain a better understanding of the disease and to assess their usefulness in glasshouse screening of germ plasm.

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MATERIALS AND METHODS

Thirty peanut genotypes were selected, on the basis of their field reactions to *P. arachidis*, to provide a wide range of resistance to the disease (Table 1). The rust scores were recorded at the ICRISAT Center over the years 1979–1982, using a nine-point scale (1 = no disease, 9 = more than 50% of foliage destroyed by the disease).

Seeds were sown in a mixture of red sandy soil and farmyard manure (4:1, v/v) in plastic pots of 15-cm diameter in the glasshouse. Four seeds were sown in each pot, and the seedlings were later thinned to two per pot. Five pots were raised for each genotype.

To obtain inoculum, uredospores taken from a single pustule on the susceptible genotype TMV 2 were used to inoculate rooted detached leaves of the same genotype in a Percival plant growth chamber, using a temperature of 25 C and a 12-hr photoperiod. Uredospores were harvested with a cyclone spore collector and suspended in sterile distilled water to which a few drops of the surfactant Tween-80 (polyoxyethylene sorbitan monooleate) had been added. The suspension was adjusted with a hemacytometer to a concentration of approximately 50,000 spores per milliliter.

Forty days after sowing, the middle leaf on the main stem of each plant was labeled and sprayed with the spore suspension, using a plastic atomizer. Following inoculation at 1700–1800 hr, the plants were placed in a polyethylene enclosure in the glasshouse, misted with water for about 24 hr, and returned to the glasshouse bench, where they were arranged in a randomized block design with five replicates of each genotype. Air temperature in the glasshouse during the trial ranged from 25 to 30 C. When watering the pots, care was taken to avoid wetting the foliage.

From 7 days after inoculation, the labeled leaves were examined daily and numbers of uredosori were recorded. When daily increase in numbers ceased, the areas of the inoculated leaves were

measured by tracing their outlines onto cards, which were cut out and measured with a leaf area meter.

At 20 and 30 days after inoculation, the leaves were scanned through a stereomicroscope (at a magnification of 70), and numbers of ruptured and unruptured uredosori were recorded.

At 30 days after inoculation, an ocular micrometer was used to measure the diameters of five randomly selected uredosori on each leaflet of the labeled leaves (ie, 20 pustules per leaf).

At 30 days after inoculation, the percentage of the area of labeled leaves having rust damage, which included yellowing and necrosis, was estimated by comparison with diagrams depicting leaves with known percentages (0.5, 1, 2, 5, 10, 20, 35, 50, 75, and 100%) of their areas affected.

From these data, the following disease characters were determined: incubation period—number of days between inoculation and appearance of 50% of the pustules; infection frequency—final number of pustules per square centimeter of leaf area; pustule diameter—mean diameter (in millimeters) of a random sample of uredosori at 30 days after inoculation; percentage pustules ruptured—mean percentage of uredosori ruptured at 20 and at 30 days after inoculation; and percentage leaf area damaged—area of inoculated leaf damaged by rust as a percentage of total leaf area at 30 days after inoculation.

RESULTS

Application of inoculum was effectively limited to the target leaves; very few uredosori developed on neighboring leaves. No evidence of a secondary cycle of infection was found.

Genotypes are listed in Table 1 in order of decreasing resistance to rust as evident from their mean field rust scores; the mean values of the disease-resistance components are presented in adjacent columns. Significant differences among peanut genotypes existed for each resistance component. As can be seen from the correlation matrix (Table 2), all the components evaluated were significantly correlated, incubation period being negatively correlated with the other resistance components, which were positively correlated with one another. Similarly, mean field rust scores were negatively correlated with incubation period and positively correlated with the other resistance components (Table 2).

For the purpose of comparison and discussion of the disease resistance components in this single-cycle infection, the genotypes were placed according to their mean field rust scores into four groups: highly resistant (scores of 2.2–2.4), resistant (scores of 2.8–3.4), moderately resistant (scores of 3.8–7.0), and susceptible (score of 9).

Genotypes within these groups showed reasonable uniformity in resistance components; however, some overlapping was found in values between adjacent groups. Incubation period decreased markedly from highly resistant to resistant to moderately resistant genotypes, but moderately resistant and susceptible groups differed little in this respect. The highly resistant genotype NC Ac 17090 had the longest incubation period. With the exception of genotype PI 393526, infection frequency was lower in the highly resistant and resistant genotypes than in the moderately resistant and susceptible

TABLE 1. Components of resistance to *Puccinia arachidis* in 30 peanut genotypes

Description of genotypes			Components of resistance						
			Rust field score[b]	Incubation period	Infection frequency	Pustule diameter	Ruptured pustules (%)[c]		Leaf area damage (%)[c]
Identity	ICG No.[a]	Botanical variety	(mean)	(days)	(lesions/cm^2)	(mm)	20 dai[d]	30 dai	
NC Ac 17090	1697	*fastigiata*	2.2	19.3	5.9	0.68	0.3	0.5	3.6
PI 405132	7897	*fastigiata*	2.4	18.3	8.1	0.63	1.3	5.6	3.9
PI 393646	7986	*fastigiata*	2.4	18.1	6.7	0.57	0.6	2.4	2.3
PI 414332	7900	*hypogaea*	2.4	14.7	4.1	0.86	1.4	0.5	2.7
PI 407454	7898	*fastigiata*	2.8	18.5	4.7	0.57	1.1	4.7	1.7
PI 414331	7899	*hypogaea*	2.8	11.9	1.4	0.57	3.8	0.0	0.9
EC 76446 (292)	2716	*fastigiata*	2.8	17.5	6.2	0.59	5.1	13.5	5.1
PI 393527-B	7892	*hypogaea*	3.0	15.9	4.2	0.51	14.4	38.8	5.0
PI 314817	7882	*fastigiata*	3.0	15.2	3.2	0.49	2.4	15.5	2.8
PI 393643	7895	*fastigiata*	3.0	14.7	5.5	0.73	3.0	9.2	4.8
PI 381622	7885	*fastigiata*	3.0	13.0	6.9	0.94	2.4	7.5	2.3
PI 350680	6340	*fastigiata*	3.0	11.3	3.6	0.79	0.3	0.5	1.9
PI 393517	7889	*fastigiata*	3.2	13.8	6.7	0.49	1.2	4.5	1.9
PI 393531	7893	*fastigiata*	3.4	11.4	4.5	0.51	0.0	2.0	1.3
NC Ac 17129	1704	*fastigiata*	3.8	11.4	21.0	1.29	95.5	100.0	13.9
NC Ac 17132	1707	*fastigiata*	3.8	9.9	12.3	1.12	96.0	100.0	20.0
PI 298115	4746	*hypogaea*	4.0	9.2	11.3	1.16	90.5	100.0	16.2
NC Ac 17130	1705	*fastigiata*	4.2	10.1	10.2	1.29	97.1	100.0	8.0
NC Ac 17124	6280	*fastigiata*	4.2	9.7	23.0	1.24	97.0	100.0	25.0
NC Ac 17127	1703	*fastigiata*	4.2	9.5	29.5	0.95	96.0	100.0	12.3
NC Ac 17135	1710	*fastigiata*	4.2	9.3	22.5	1.31	95.7	100.0	21.0
PI 393526	7890	*fastigiata*	4.2	9.8	6.1	1.04	94.8	100.0	6.3
NC Ac 17142	1712	*fastigiata*	5.4	9.6	11.8	1.10	95.5	100.0	12.3
C. No. 45-23	3580	*fastigiata*	5.6	10.2	9.2	1.07	100.0	100.0	15.8
PI 270806	6330	*hypogaea*	7.0	9.3	15.8	1.35	100.0	100.0	24.2
J 11[e]	1326	*vulgaris*	9.0	9.7	16.4	1.15	100.0	100.0	27.5
TMV 2[e]	221	*vulgaris*	9.0	9.3	13.5	1.12	100.0	100.0	18.1
NC 3033[e]	6446	*hypogaea*	9.0	9.1	10.8	1.01	100.0	100.0	15.3
EC 76446[e]	4580	*vulgaris*	9.0	9.0	14.9	1.26	99.6	100.0	24.5
Robut 33-1[e]	799	*hypogaea*	9.0	9.0	15.5	1.08	99.8	100.0	19.5
WDLSD[f]	$p = 0.05$		0.49	1.12	3.42	0.104	4.83	4.63	4.11
	$p = 0.01$		0.65	1.46	4.46	0.136	6.31	6.05	5.37

[a] ICRISAT groundnut accession number.
[b] Mean rust scores recorded at the ICRISAT Center over the years 1979–1982, using a nine-point disease scale (1 = no disease, 9 = more than 50% of foliage destroyed by the disease).
[c] Actual figures; analysis was done after arc sine transformation.
[d] Days after inoculation.
[e] Standard susceptible genotypes.
[f] Waller and Duncan's Bayesian least significant differences.

TABLE 2. Correlation[a] coefficients[b] for components of resistance to *Puccinia arachidis* studied in the glasshouse and for rust field score

Character	Character									
	1	2	3	4	5	6	7	8	9	10
1. Rust field score	1.000	−0.654	0.438[c]	0.580	0.707	0.747	0.681	0.679	0.738	0.722
2. Incubation period		1.000	−0.574	−0.775	−0.831	−0.826	−0.812	−0.799	−0.706	−0.705
3. Infection frequency			1.000	0.707	0.745	0.732	0.740	0.740	0.755	0.777
4. Pustule diameter				1.000	0.893	0.883	0.875	0.866	0.826	0.849
5. Ruptured pustules (%), 20 dai[d]					1.000	0.996	0.994	0.990	0.864	0.899
6. Ruptured pustules (%), 20 dai T[e]						1.000	0.991	0.988	0.878	0.911
7. Ruptured pustules (%), 30 dai[d]							1.000	0.998	0.855	0.897
8. Ruptured pustules (%), 30 dai T[e]								1.000	0.853	0.897
9. Leaf area damage (%)									1.000	0.990
10. Leaf area damage (%), T[e]										1.000

[a]The correlation between rust field score and any component of rust resistance studied in the glasshouse is based on 30 observations; the correlation between any two components of rust resistance is based on 150 observations.
[b]Significant at the 1% level, except as noted.
[c]Significant at the 5% level.
[d]Days after inoculation.
[e]After arc sine transformation.

genotypes. The highly resistant and resistant genotypes had much smaller uredosori than did moderately resistant and susceptible genotypes. In general, no significant differences were found in pustule diameter between the moderately resistant and susceptible genotypes. Highly resistant and resistant genotypes had very few uredosori ruptured at 20 or at 30 days after inoculation. Microscopic examination of uredosori that had failed to rupture showed that uredospores had been formed. All moderately resistant and susceptible genotypes had over 90% of uredosori ruptured at 20 days after inoculation and 100% ruptured at 30 days after inoculation. The percentage of leaf area damaged was low in highly resistant and resistant genotypes and high in the susceptible genotypes, in which there was considerable chlorosis and necrosis of leaf tissues. As expected with a single cycle of infection, percentage leaf area damaged was closely linked to infection frequency. The genotype NC Ac 17127, which had the highest infection frequency (29.5), had a relatively low percentage of leaf area damaged (12.3), but this cultivar also had the smallest uredosori of all genotypes in the moderately resistant group.

DISCUSSION

Subrahmanyam et al (23) and Nevill (13), working with genotypes of both the cultivated peanut and the wild *Arachis* species, showed that rust resistance was not correlated with either frequency or size of stomata. Irrespective of whether a genotype was immune, resistant, or susceptible, the uredospores germinated on the leaf surfaces and the fungus entered the leaf through the stomata. In some immune species of *Arachis*, the mycelium died shortly after entry. Differences in resistance were manifested through differences in rate and extent of mycelial development within the substomatal cavity and in invasion of the leaf tissues. Cook (3) suggested that rust resistance in some peanut genotypes was mainly physiologic, resulting in necrotic lesions or poorly sporulating uredosori. She found that leaves of greenhouse-grown plants, particularly those of resistant genotypes, showed a decline in susceptibility to infection with age, and she related this to a corresponding decrease in leaf wettability (4). She also suggested the use of differential leaf wettability as a preliminary screening technique for selecting genotypes resistant to peanut rust when physiological resistance was not being investigated (5). Subrahmanyam et al (22), working with four peanut genotypes in the glasshouse, also reported a decline in susceptibility to rust as the leaves aged.

The present study has shown that rust resistance in peanut genotypes is associated with a failure of the fungus to successfully invade the host tissues at all infection sites, resulting in a low infection frequency. Even if invasion is successful, the host reaction slows down disease development, giving an increased incubation period and slowing down or inhibiting the development of uredosori and the release of uredospores. This kind of reaction to disease is characteristic of "horizontal resistance" (24,25) and is similar to the "slow rusting" or "partial resistance" reported by several investigators of cereal rusts (8–12,14–19).

Ohm and Shaner (14) and Kuhn et al (9), working with wheat rust, suggested that a linkage or the pleiotropic effects of genes controlling resistance components could explain the close association of the individual components. This could well be the explanation of the correlation observed between the components of resistance to rust in peanuts.

The effects of individual components of resistance on an epidemic are difficult to interpret because the components interact and their effects are cumulative over the course of the epidemic (18,19). In the present experiment, the components were examined in a single cycle of infection, which did not permit measurement of their effects on disease development through further cycles of infection. The field rust scores quoted were taken toward the end of rust epidemics and should reflect the interaction of the resistance components and their effects through many infection cycles. However, the resistant genotypes had in all cases been grown in the presence of "infector rows" and check plots of susceptible genotypes and were therefore subjected to abundant external inoculum throughout the season. This has no doubt previously led to underestimation of the magnitude of resistance, as has been shown for similar situations (24).

It would be interesting to see whether the genotypes PI 414331, PI 350680, and PI 393531, which had shorter incubation periods than other genotypes in the resistant group, would maintain this position if grown in isolation with only one initial inoculation with *P. arachidis*.

The significant relationship between field rust scores and resistance components measured in the glasshouse indicate that the latter could be used in resistance screening to separate highly resistant and resistant from susceptible genotypes. They would be less useful in classification of genotypes with moderate levels of resistance, but they do provide a means to measure rate-reducing resistance, which is difficult to measure in the field because of interplot interference and the preponderance of alloinfections. Notwithstanding this reservation, glasshouse screening of germ plasm by measuring resistance components could have practical application in areas where rust epidemics do not occur or are of irregular occurrence or where the presence of other diseases complicates field disease scoring.

LITERATURE CITED

1. Bromfield, K. R. 1974. Current distribution of rust of groundnut and known sources of resistance. FAO Plant Prot. Bull. 22:29-31.
2. Bromfield, K. R., and Cevario, S. J. 1970. Greenhouse screening of peanut (*Arachis hypogaea*) for resistance to peanut rust (*Puccinia arachidis*). Plant Dis. Rep. 54:381-383.
3. Cook, M. 1972. Screening of peanut for resistance to peanut rust in the greenhouse and field. Plant Dis. Rep. 56:382-386.

4. Cook, M. 1980. Host-parasite relations in uredial infections of peanut by *Puccinia arachidis*. Phytopathology 70:822-826.
5. Cook, M. 1980. Peanut leaf wettability and susceptibility to infection by *Puccinia arachidis*. Phytopathology 70:826-830.
6. Gibbons, R. W. 1980. Groundnut improvement research technology for the semi-arid tropics. Pages 27–37 in: Proc. Int. Symp. Development and Transfer of Technology for Rainfed Agriculture and the SAT Farmer, 28 August–1 September 1979. Int. Crops Res. Inst. Semi-Arid Trop. Patancheru, Andhra Pradesh, India.
7. Hammons, R. O. 1977. Groundnut rust in the United States and the Caribbean. PANS 23:300-304.
8. Hooker, A. L. 1967. The genetics and expression of resistance in plants to rusts of the genus *Puccinia*. Annu. Rev. Phytopathol. 5:163-182.
9. Kuhn, R. C., Ohm, H. W., and Shaner, G. 1980. Inheritance of slow leaf-rusting resistance in Suwon 85 wheat. Crop Sci. 20:655-659.
10. Mackenzie, D. R. 1976. Application of two epidemiological models for the identification of slow stem rusting in wheat. Phytopathology 66:55-59.
11. Milus, E. A., and Line, R. F. 1980. Characterization of resistance to leaf rust in Pacific Northwest wheats. Phytopathology 70:167-172.
12. Neervoort, W. J., and Parlevliet, J. E. 1978. Partial resistance of barley to leaf rust, *Puccinia hordei*. V. Analysis of the components of partial resistance in eight barley cultivars. Euphytica 27:33-39.
13. Nevill, D. J. 1980. Studies of resistance to foliar pathogens. Pages 199–202 in: Proc. Int. Workshop on Groundnuts, 13–17 October 1980. Int. Crops Res. Inst. Semi-Arid Trop. Patancheru, Andhra Pradesh, India.
14. Ohm, H. W., and Shaner, G. 1976. Three components of slow leaf-rusting at different growth stages in wheat. Phytopathology 66:1356-1360.
15. Parlevliet, J. E. 1976. Evaluation of the concept of horizontal resistance in the barley/*Puccinia hordei* host-pathogen relationship. Phytopathology 66:494-497.
16. Parlevliet, J. E., and Zadoks, J. C. 1977. The integrated concept of disease resistance: A new view including horizontal and vertical resistance in plants. Euphytica 26:5-21.
17. Shaner, G., and Finney, R. E. 1980. New sources of slow leaf rusting resistance in wheat. Phytopathology 70:1183-1186.
18. Shaner, G., and Hess, F. D. 1978. Equations for integrating components of slow leaf-rusting resistance in wheat. Phytopathology 68:1464-1469.
19. Shaner, G., Ohm, H. W., and Finney, R. E. 1978. Response of susceptible and slow leaf-rusting wheats to infection by *Puccinia recondita*. Phytopathology 68:471-475.
20. Smith, D. H. 1979. Disease resistance in peanuts. Pages 431–447 in: Biology and Breeding for Resistance to Arthropods and Pathogens in Agricultural Plants. M. K. Harris, ed. Proc. Int. Short Course in Host Plant Resistance, 22 July–4 August 1979. Texas A&M University, College Station.
21. Subrahmanyam, P., Reddy, D. V. R., Gibbons, R. W., Rao, V. R., and Garren, K. H. 1979. Current distribution of groundnut rust in India. PANS 25:25-29.
22. Subrahmanyam, P., Gibbons, R. W., Nigam, S. N., and Rao, V. R. 1980. Screening methods and further sources of resistance to peanut rust. Peanut Sci. 7:10-12.
23. Subrahmanyam, P., Mehan, V. K., Nevill, D. J., and McDonald, D. 1980. Research on fungal diseases of groundnut at ICRISAT. Pages 193–198 in: Proc. Int. Workshop on Groundnuts, 13–17 October 1980. Int. Crops Res. Inst. Semi-Arid Trop. Patancheru, Andhra Pradesh, India.
24. Vanderplank, J. E. 1963. Plant Diseases: Epidemics and Control. Academic Press, New York. 349 pp.
25. Vanderplank, J. E. 1968. Disease Resistance in Plants. Academic Press, New York. 206 pp.