Ann appl Biol (1990), 117, 379–384
Printed in Great Britain

The nature of the resistance in groundnut to rosette disease

By K. R. BOCK*, A. F. MURANT† and R. RAJESHWARI**

**International Crops Research Institute for the Semi-Arid Tropics, Chitedze Agricultural Research Station, Private Bag 63 Lilongwe, Malawi*

†Scottish Crop Research Institute, Invergowrie, Dundee, Scotland DD2 5DA, UK

(Accepted 14 June 1990)

Summary

Groundnut rosette disease is caused by a complex of three agents, groundnut rosette virus (GRV) and its satellite RNA, and groundnut rosette assistor virus (GRAV); the satellite RNA is mainly responsible for the disease symptoms. Groundnut genotypes possessing resistance to rosette disease were shown to be highly resistant (though not immune) to GRV and therefore to its satellite RNA, but were fully susceptible to GRAV.

Key words: Groundnut rosette disease, resistance, groundnut rosette virus, groundnut rosette assistor virus, groundnut rosette virus, satellite, peanut

Introduction

Rosette (Zimmermann, 1907; Storey & Bottomley, 1928; Storey & Ryland, 1957) is the most important virus disease of groundnut (*Arachis hypogaea*) in Africa, but is not known to occur in other groundnut-growing areas of the world (Gibbons, 1977; Reddy & McDonald, 1988). Two main forms of the disease are distinguished (Hayes, 1932; Smartt, 1961; Hull & Adams, 1968): chlorotic rosette, which is reported from most African countries south of the Sahara, and green rosette, which is reported only from West Africa and Uganda. Resistance to rosette was first found in groundnut germplasm originating from adjacent regions of Burkino Faso and Côte d'Ivoire (Sauger & Catharinet, 1954*a*,*b*; De Berchoux, 1958) and material from this region was the source of resistance for all rosette-resistant cultivars developed subsequently (Dhéry & Gillier, 1971). The resistance is governed by two independent recessive genes (De Berchoux, 1960; Nigam & Bock, 1990) and seems to be effective against both chlorotic rosette (De Berchoux, 1960) and green rosette (Harkness, 1977).

The aetiology of rosette disease is complex. Diseased groundnut plants contain groundnut rosette virus (GRV), usually accompanied by groundnut rosette assistor virus (GRAV), on which GRV depends for transmission by the aphid *Aphis craccivora* (Hull & Adams, 1968). GRAV is a luteovirus (Casper *et al.*, 1983; Reddy *et al.*, 1985*a*) and is not transmissible by

Submitted as Journal Article No. 1015 by the International Crops Research Institute for the Semi-Arid Tropics. The work was supported in part by the Overseas Development Administration (Research Project R4148).

**On leave from the International Crops Research Institute for the Semi-Arid Tropics, Patancheru PO, Andhra Pradesh 502 324, India. Present address: School of Life Sciences, Central University of Hyderaba Andhra Pradesh 500 134, India

manual inoculation; it has been purified and a polyclonal antiserum is available (Rajeshwari & Murant, 1988). GRV is transmissible manually, but no virus-like particles have been observed in plant extracts or in partially purified preparations (Reddy *et al.*, 1985*b*).

Groundnut plants infected by GRAV alone are symptomless. Rosette symptoms in groundnut are associated with infection by GRV but are caused not by GRV itself but by a satellite RNA that depends on GRV for its replication (Murant, Rajeshwari, Robinson & Raschké, 1988). Satellite-free cultures of GRV induce no symptoms in groundnut or only a transient mottle. Different forms of the satellite RNA are responsible for the chlorotic and green forms of rosette (Murant & Kumar, 1989, 1990). Other forms of the satellite RNA infect groundnut without causing rosette. Thus groundnut plants that show no symptoms may nevertheless be infected by one or more components of the virus complex. This paper describes the nature of the resistance in several rosette-resistant groundnut genotypes.

Materials and Methods

Groundnut genotypes and inoculation methods. In the glasshouse experiments the control susceptible groundnut cultivar was Spancross and the resistant genotypes were RG1, RMP40, RMP91, RMP93, RRI/6 and RRI/24. Seedlings were raised at Chitedze in aphid-proof glasshouses equipped with extractor fans to keep the temperatures as close as possible to ambient. For aphid-inoculation tests, a viruliferous culture of *Aphis craccivora* was maintained on Spancross groundnut infected with a chlorotic form of rosette (i.e. a field-derived culture of the virus complex containing GRAV plus GRV together with its satellite RNA). Batches of 10-15 aphids were placed on test groundnut seedlings, allowed to feed for 7 days, and then killed by spraying with pirimiphos-methyl. This inoculation was repeated twice during the following 2 months. For graft-inoculation tests, resistant or susceptible scions were top-grafted onto rosette-diseased Spancross stocks.

In the field experiments, the control susceptible cultivar was Malimba; the resistant genotypes were those listed above with the exception of RMP91 and the addition of 48-21, RMP49 and ICGV-SM. The material was planted in 6 m rows, two rows of a resistant line followed by a single row of Malimba, and so on. Rosette disease was introduced into the experimental plots by the procedure of Nigam & Bock (1990): about 1 wk after the emergence of the seedlings, rosette-diseased glasshouse-grown Spancross plants, heavily infested with *A. craccivora*, were transplanted at 1.5 m intervals into the rows of Malimba. In addition, the whole area of the experiment was randomly seeded with viruliferous aphids from the glasshouse culture. The incidence of rosette disease in the plots was recorded at the end of the season.

Virus detection. To determine the virus content of groundnut test seedlings, samples were sent to SCRI, Dundee, under licences issued by the Department of Agriculture for Scotland. GRAV was detected by double antibody sandwich ELISA (DAS-ELISA; Clark & Adams, 1977), in which GRAV polyclonal antiserum (Rajeshwari & Murant, 1988) was used as both first (plate-coating) and second (detecting) antibody, or by triple antibody sandwich ELISA (TAS-ELISA; Martin & Stace-Smith, 1984), in which polyclonal antisera to GRAV, potato leafroll virus (PLRV) or beet western yellows virus were used as the first antibody and a monoclonal antibody (MAb) to PLRV, called SCR6, was used as the second antibody (Rajeshwari, Murant & Massalski, 1987). GRV was detected by grinding groundnut leaves in 10 mM tris-HCl buffer, pH 8.0, containing 20 mM sodium sulphite and rubbing the extracts on Corundum-dusted leaves of *Chenopodium amaranticolor, Nicotiana benthamiana* and *N.*

clevelandii (Reddy *et al.*, 1985*b*). Representative GRV isolates were examined for the presence of satellite RNA by electrophoretic analysis of dsRNA extracted from infected *N. benthamiana*, as described by Murant *et al.* (1988)

Results

Table 1*a* shows the responses of resistant and susceptible genotypes to inoculation by aphids in a glasshouse experiment at Chitedze. All inoculated plants of the susceptible cultivar Spancross developed obvious rosette symptoms within 18 days after the first aphid inoculation, but no symptoms were observed at this time in any plants of the six resistant lines. In tests made 56-70 days after inoculation, all plants of all the genotypes were found to contain GRAV; GRV was found only in the four plants of the susceptible genotype Spancross and in one plant of the resistant genotype RRI/6. This RRI/6 plant eventually developed rosette symptoms between 75 and 96 days after the first inoculation.

Electrophoretic analysis showed that the GRV isolates recovered in *N. benthamiana* from the four plants of cv. Spancross and one of cv. RRI/6 all contained the satellite RNA. No dsRNA bands were detected in preparations made from symptomless *N. benthamiana* inoculated with extracts from resistant genotypes. Electrophoresis of dsRNA preparations made from 10 g composite leaf samples from the symptomless groundnut plants of the resistant genotypes revealed very faint bands in the position expected for the satellite RNA in RMP 93, RRI/6 and RRI/24; however, this is not thought to indicate that GRV is present at low levels in these resistant plants because similar very faint bands were found in dsRNA extracts from healthy groundnut plants and from groundnut plants infected with GRAV alone. No

Table 1. *Response of rosette-resistant (R) and rosette-susceptible (S) cultivars of groundnut to inoculation with the components of rosette disease*

a) Expt 1 (aphid-inoculated in the glasshouse)

Cultivar	No. plants rosetted/ no. inoculated	Days to symptom appearance	No. plants infected/ no. tested	
			GRAV	GRV
Spancross (S)	4/4	12-18	4/4	4/4
RG1 (R)	0/5	—	5/5	0/5
RMP40 (R)	0/5	—	5/5	0/5
RMP91 (R)	0/5	—	5/5	0/5
RMP93 (R)	0/5	—	5/5	0/5
RRI/6 (R)	1/5	96	5/5	1/5
RRI/24 (R)	0/4	—	4/4	0/5

b) Expt 2 (graft-inoculated in the glasshouse)

Cultivar	No. plants rosetted/ no. inoculated	Days to symptom appearance	No plants infected/ no. tested			
			Rosetted		Not rosetted	
			GRAV	GRV	GRAV	GRV
Spancross (S)	10/10	22-29	2/3	3/3	—	—
RG1 (R)	6/11	84-137	3/3	2/3	1/5	0/5
RMP40 (R)	5/9	113-140	3/3	3/3	4/4	0/4
RMP91 (R)	3/9	71-154	2/3	2/3	6/6	0/6
RMP93 (R)	8/10	77-128	2/3	3/3	1/2	0/2
RRI/6 (R)	6/10	92-133	2/3	3/3	1/4	0/4
RRI/24 (R)	3/10	77-135	2/3	3/3	3/6	0/6

Table 2. *Incidence of rosette disease in rosette-resistant (R) and rosette-susceptible (S) groundnut genotypes exposed in the field to large populations of viruliferous* Aphis craccivora

Genotype	No. plants with rosette no. exposed				
	1985/86	1986/87	1988/89	Total	%
Malimba (S)	9474/9935	567/580	577/622	10618/11137	95·3
48-21 (R)	—	0/85	—	0/85	0
ICGV-SM (R)	—	—	1/558	1/558	0·2
RG1 (R)	0/85	0/350	50/2440	50/2875	1·74
RMP40 (R)	0/93	0/218	0/165	0/476	0
RMP49 (R)	—	0/80	—	0/80	0
RMP93 (R)	—	2/83	—	2/83	2·4
RRI/6 (R)	—	0/195	5/162	5/357	1·4
RRI/24 (R)	—	—	3/154	3/154	1·9

Fig. 1. Part of a field trial at Chitedze, Malawi, in the 1988/1989 growing season, showing paired rows of rosette-resistant lines on either side of a single row of the control susceptible cultivar Malimba (centre). All the plants were exposed to infection by the release of viruliferous *A. craccivora*.

symptoms of rosette developed in healthy Spancross shoots which were grafted on to these resistant plants 5 wk after the original inoculation, although similar shoots grafted on to rosetted Spancross plants showed rosette symptoms within 17 days.

In a second glasshouse experiment (Table 1*b*), healthy scions of each genotype were grafted to fully rosetted plants of cv. Spancross. All scions of cv. Spancross developed severe rosette within 22-29 days, whereas none of the resistant scions showed rosette until 71 days after grafting and almost half remained symptomless for the duration of the experiment (6 months). In those resistant scions that developed symptoms the majority of side shoots remained symptomless but a few developed mild rosette. GRAV was detected in 30/45 resistant scions (whether showing rosette symptoms or not), whereas GRV was found in 16/18 scions with symptoms, and in none of 27 without symptoms. All of six tested GRV isolates from resistant plants contained the satellite RNA.

Further evidence that GRAV was present in the resistant lines was obtained when *A. craccivora* was shown to transmit GRV readily from 14/16 of the resistant plants that eventually showed rosette symptoms.

Table 2 shows the responses of resistant and susceptible genotypes in field experiments (Fig. 1) conducted at Chitedze during the 1985/86, 1986/87 and 1988/89 groundnut-growing seasons. Although small numbers of infections were observed in several of the resistant genotypes, the greatest incidence of rosette observed was 2.4%, compared with 95.3% in the susceptible cv. Malimba. No tests were made for GRAV infection in the resistant plants in these experiments. However, in a separate field experiment with cv. RG1, in which rows of healthy plants alternated with rows of plants that had been infected with GRAV by aphid inoculation from the stock glasshouse rosette culture, 56/80 (70%) of the initially healthy plants were found by ELISA to be infected with GRAV at the end of the growing season. None of the plants showed symptoms of rosette.

Discussion

The results presented here show that rosette-resistant groundnut genotypes are susceptible to GRAV, and become readily infected with it in the field, but are highly resistant to GRV and therefore to the GRV satellite RNA which is the actual cause of rosette symptoms (Murant *et al.*, 1988; Murant & Kumar, 1989, 1990). However, our results confirm previous reports (Sauger & Catharinet, 1954*a*,*b*; De Berchoux, 1960; Nutman, Roberts & Williamson, 1964) that the resistance of these genotypes to rosette is not absolute: although most plants remained free from rosette in the field, even when exposed to high levels of infection with viruliferous aphids, a small proportion (up to about 2%) became diseased, and the plants that succumbed were found to contain GRV along with its satellite RNA.

A considerably greater proportion of infections (albeit late in onset) was observed in the glasshouse experiment in which resistant genotypes were grafted on to susceptible rootstocks. This suggests that the resistance tends to break down under high inoculum pressure or adverse environmental conditions. These factors deserve fuller investigation: although there is no evidence that they are important under field conditions at Chitedze, they may perhaps be important in other parts of Africa.

Acknowledgements

We wish to acknowledge the valuable assistance of Mr J. B. Kamangira with the field and glasshouse experiments in Malawi.

References

Casper, R., Meyer, S., Lesemann, D.-E., Reddy, D.V.R., Rajeshwari, R., Misari, S. M. & Subbarayadu, S. S. (1983). Detection of a luteovirus in groundnut rosette diseased groundnuts (*Arachis hypogaea*) by enzyme-linked immunosorbent assay and immunoelectron microscopy. *Phytopathologische Zeitschrift* **108**, 12-17.

Clark, M. F. & Adams, A. N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for detection of plant viruses. *Journal of General Virology* **34**, 475-484.

De Berchoux, C. (1958). Etude sur la résistance de l'arachide à la rosette en Haute-Volta. Premiers résultats. *Oléagineux* **13**, 237-239.

De Berchoux, C. (1960). La rosette de l'arachide en Haute-Volta Comportement des lignées résistantes. *Oléagineux* **15**, 229-233.

Dhéry, M. & Gillier, P. (1971) Un nouveau pas dans la lutte contre la rosette de l'arachide Resultats obtenus en Haute-Volta avec les nouveaux hybrides. *Oléagineux* **26**, 243-251.

Gibbons, R. W. (1977). Groundnut rosette virus. In *Diseases, Pests and Weeds in Tropical Crops*, pp. 19-21. Eds J. Kranz, H. Schmutterer and W. Koch. Berlin: Paul Parey.

Harkness, C. (1977). The breeding and selection of groundnut varieties for resistance to rosette virus disease in Nigeria. In *Submission to the African Groundnut Council, June 1977, Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria*, pp. 1-45.

Hayes, T. R. (1932). Groundnut rosette disease in the Gambia. *Tropical Agriculture, Trinidad* **9**, 211-217.

Hull, R. & Adams, A. N. (1968). Groundnut rosette and its assistor virus *Annals of Applied Biology* **62**, 139-145.

Martin, R. R. & Stace-Smith, R. (1984). Production and characterization of monoclonal antibodies specific to potato leaf roll virus. *Canadian Journal of Plant Pathology* **6**, 206-210

Murant, A. F. & Kumar, I. K. (1989). Role of a satellite RNA in green and chlorotic forms of groundnut rosette. *Abstracts 4th International Plant Virus Epidemiology Workshop, Montpellier, 1989*, 259-260.

Murant, A. F. & Kumar, I. K. (1990). Different variants of the satellite RNA of groundnut rosette virus are responsible for the chlorotic and green forms of groundnut rosette disease. *Annals of Applied Biology* **117**, 85–92.

Murant, A. F., Rajeshwari, R., Robinson, D. J. & Raschké, J. H. (1988) A satellite RNA of groundnut rosette virus that is largely responsible for symptoms of groundnut rosette disease. *Journal of General Virology* **69**, 1479-1486.

Nigam, S. N. & Bock, K. R. (1990). Inheritance of resistance to groundnut rosette virus in groundnut (*Arachis hypogaea*). *Annals of Applied Biology* **117**, (in press).

Nutman, F. J., Roberts, F. M. & Williamson, J. G. (1964). Studies on varietal resistance in the groundnut, (*Arachis hypogaea* L.) to rosette disease. *Rhodesian Journal of Agricultural Research* **2**, 63-77.

Rajeshwari, R. & Murant, A. F. (1988). Purification and particle properties of groundnut rosette assistor virus, and production of a specific antiserum. *Annals of Applied Biology* **112**, 403-414.

Rajeshwari, R., Murant, A. F. & Massalski, P. R. (1987). Use of monoclonal antibody to potato leafroll virus for detecting groundnut rosette assistor virus by ELISA. *Annals of Applied Biology* **111**, 353-358.

Reddy, D. V. R. & McDonald, D. (1988). Research on groundnut virus diseases in the semi-arid tropics. In *Coordinated Research on Groundnut Rosette Virus Disease: Summary Proceedings of the Consultative Group Meeting, 8-10 March 1987, Lilongwe, Malawi*, pp. 4-6. Eds D. McDonald, D. V. R. Reddy, J. A. Wightman and S. N. Nigam. Patancheru, India: ICRISAT.

Reddy, D. V. R., Murant, A. F., Duncan, G. H., Ansa, O. A., Demski, J. W. & Kuhn, C. W. (1985*a*). Viruses associated with chlorotic rosette and green rosette diseases of groundnut in Nigeria. *Annals of Applied Biology* **107**, 57-64.

Reddy, D. V. R., Murant, A. F., Raschké, J. H., Mayo, M. A. & Ansa, O. A. (1985*b*). Properties and partial purification of infective material from plants containing groundnut rosette virus. *Annals of Applied Biology* **107**, 65-78.

Sauger, L. & Catharinet, M. (1954*a*). La rosette chlorotique de l'arachide et les lignées sélectionnées. *L'Agronomie Tropicale* **9**, 28-36.

Sauger, L. & Catharinet, M. (1954*b*). Nouvelles observations sur la rosette chlorotique de l'arachide et les lignées sélectionnées. *Annales du Centre de Recherches Agronomiques de Bambey au Sénégal. Année 1953. Bulletin Agronomique* **11**, 204-216

Smartt, J. (1961). The diseases of groundnuts in Northern Rhodesia. *Empire Journal of Experimental Agriculture* **29**, 79-87.

Storey, H. H. & Bottomley, A. M. (1928). The rosette disease of peanuts (*Arachis hypogaea* L.). *Annals of Applied Biology* **15**, 26-45.

Storey, H. H. & Ryland, A. K. (1957) Virus causing rosette and other diseases in groundnuts. *Annals of Applied Biology* **45**, 318-326.

Zimmermann, A. (1907). Über eine Krankheit der Erdnusse (*Arachis hypogaea*). *Der Pflanzer* **3**, 129-133.

(Received 17 April 1990)