Methodology of Groundnut Rosette Resistance Screening and Vector-Ecology Studies in Malawi

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The challenge in the selection of acceptable groundnut rosette virus (GRV) resistant cultivars lies not with the generation of resistant × susceptible crosses, but in the effective screening of very large numbers of hybrids that the breeding program demands. Groundnut rosette is a disease which, though devastating, is sporadic in occurrence in southern Africa, often with intervals of several years between pandemics. Reliance cannot, therefore, be placed on natural incidence when screening crosses, and an alternative strategy must be evolved. The development of disease nurseries is one such means, and we report our progress in this direction. We remain ignorant of the seasonal origins of GRV, the resolution of which must involve studies on the ecology of the vector, *Aphis craccivora* Koch.

Methodology of GRV-Resistance Screening

We have developed a satisfactory technique for GRV-resistance screening which involves the management of a field disease nursery during the rainy season and subsequent controlled greenhouse screening tests of apparently healthy field survivors.

We base our field nursery management on the GRV's pattern of spread in Malawi, where only primary infections give rise to typical patches of the disease.

At normal sowing time, generally at the onset of the rains, we plant one infector row of a susceptible variety (Malimba) between two contiguous rows of test lines. Previous to this period, we raise large numbers of susceptible seedlings in the greenhouse, inoculate them with GRV, and allow dense populations of viruliferous apterae to develop on the infected plants. About 1 week after seedling emergence, we transplant, at 1.5-m spacing in each of the infector rows, the diseased seedlings still heavily infested with vectors. We subsequently continue to harvest viruliferous aphids from greenhouse cultures and seed the nursery with them on many occasions. This resulted in a 90% incidence in 1984/85 (2.0-m spacing between infected transplants) and a 98% incidence in 1985/86 (1.5-m spacing between infected transplants) in the infector rows.

In 1985/86, when some 29 000 test plants from crosses between susceptible and resistant parents and from backcrosses were screened, the apparently healthy survivors consisted of a mixture of susceptible 'escapes' and plants that were homozygous for resistance (Table 1). 'Escapes' are screened out by greenhouse tests during the ensuing dry season. Agreement between observed and predicted numerical values for resistance among the progenies of resistant × susceptible parents and of backcrosses indicates the double-recessive nature of GRV resistance (Table 2).

Studies on Resistance: Grafting and Other Experiments

Mrs R. Rajeshwari and Dr A.F. Murant tested graft inoculated resistant plants from Malawi for the presence of the groundnut rosette assistor virus (GRAV) by means of Enzyme-Linked Immunosorbent Assay (ELISA), and for GRV by sap inoculation to *Chenopodium amaranticolor* and *Nicotiana benthamiana*.

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Table 1. Incidence of groundnut rosette virus (GRV) in all susceptible, resistant, and susceptible × resistant (S × R) tested at the field screening nursery, Chitedze, Malawi, 1985/86.

Type of line	Number of plants infected	Number of plants exposed	Rosette disease incidence (%)	
			Observed	Expected
Susceptible 'spreader' rows	20212	20680	97.7	100
Susceptible parents (S)	209	217	96.3	100
Resistant parents (R)	0	174	0.0	0
S × R crosses:				
F_1	76	79	96.2	100
F_2	2367	25927	91.3	93[1]
Backcrosses:				
(S × R) × S	1387	1444	96.1	100
(S × R) × R	1382	1899	72.8	75[2]

1. Predicted ratio = 1 resistant to 15 susceptible plants.
2. Predicted ratio = 1 resistant to 3 susceptible plants.

Table 2. Data for groundnut rosette virus (GRV) inheritance studies only: GRV susceptibility in susceptible × resistant (S × R) crosses, Chitedze, Malawi, 1985/86[1].

Type of line	Number of plants infected	Number of plants exposed	Rosette disease incidence (%)	
			Observed	Expected
S × R crosses:				
F_1 (R × S)	21	23		
S × R	30	30		
Total	51	53	96.2	100
F_2 (R × S)	4537	4791		
S × R	2728	2971		
Total	7265	7662	94.3	93.8[2]
Backcrosses:				
(R × S) × R	650	846		
(S × R) × R 348 × 457				
Total	998	1303	76.6	75[3]
(R × S) × S	865	873		
(S × R) × S	482	482		
Total	1347	1355	99.4	100

1. Results include greenhouse retests on apparently healthy survivors of field tests.
2. Predicted ratio = 1 resistant to 15 susceptible plants.
3. Predicted ratio = 1 resistant to 3 susceptible plants.

In Malawi, we inoculated seedlings of resistant varieties, RG 1, RMP 40, RMP 90, RMP 93, RR1/6, RR1/24 thrice, using batches of 20 viruliferous aphids. After 5 weeks, the resistant plants were top-grafted with healthy, susceptible shoots. As controls, we grafted healthy susceptible shoots into rosetted plants. these always developed GRV within 17 days of grafting, whereas no healthy scions grafted onto resistant inoculated plants developed symptoms of GRV. In a second experiment, we grafted healthy resistant shoots into fully rosetted plants. These grew well, produced side shoots, and behaved in one of three following ways:

1. Some of them remained free of symptoms for the duration of the experiment (6 months). Healthy susceptible scions grafted into these developed GRV disease, which was readily transmitted to healthy susceptible seedlings by the vector.
2. In others, the majority of side shoots of the scion remained symptomless, but often one or two of those nearest to the graft union developed suppressed or muted GRV-disease symptoms.
3. In very few grafts, the resistant scions developed more or less severe symptoms of GRV disease with severely shortened internodes.

These variations in reaction by the resistant shoots of essentially similar, if not identical, genotypes to continuous infection with virus is not understood, but the graft experiments indicate that the resistant varieties studied are all highly resistant (almost to the point of immunity) to inoculation of GRV by the vector. However, they are not immune to GRV. When infected by grafting, GRV symptoms are either completely suppressed or greatly muted, and only rarely do typical symptoms appear.

In a third series of experiments, we sent shoots of heavily inoculated, resistant varieties to Dr Murant at the Scottish Crop Research Institute. All inoculated plants of all resistant varieties contained groundnut rosette assistor virus (GRAV), which was readily transmitted to groundnut seedlings by *A. craccivora*. Genes conferring resistance to GRV in the cultivated groundnut, therefore, do not also confer resistance to GRAV.

Studies on Vector Ecology

We continue to study the vector using yellow water traps, bait plants, and dry-season bait plots.

All these methods indicate the continuous presence of *A. craccivora* throughout the year, including all months of the dry season. The dry-season population, however, apparently does not carry GRV. At the onset of the rains, the population migrating into the emerging groundnut crop contains a proportion of viruliferous individuals. Table 3 summarizes early rains observations on vector and virus, from 1983/84 to 1986/87 seasons.

Table 3. Relationship of emergence of crop to arrival of viruliferous alates and development of groundnut rosette virus (GRV), Chitedze, Malawi, 1983/84 to 1986/87.

Date(s)/duration	1983/84	1984/85	1985/86	1986/87
Date of approximate onset of rains	18 Dec	6 Nov	7 Nov	1 Dec
Dates of emergence of crop	28-31 Dec	26-29 Nov	30 Nov	17 Dec
Date when first alates were seen	4 Jan	7 Dec	5 Dec	18 Dec
Date when first few GRV symptoms were observed	18 Jan	20 Dec	19 Dec	8 Jan
Number of days between emergence and first few symptoms	19-21	21-24	20	22

Based on our own observations and the results of discussions with groundnut scientists working in the region, we do not think that volunteer plants are significantly involved in the maintenance of virus or vector during the dry season in Malawi.

We deduce a sequential movement of *A. craccivora* from plant host to plant host, as these become attractive in turn to the vector during the dry season. These dry-season hosts are not necessarily GRV reservoirs. We think that, at the beginning of the rains, one or more species of plants, which are hosts of the virus are briefly colonized by the vector just prior to its infestation of the emerging groundnut crop.