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# Inheritance of resistance to groundnut rosette virus in groundnut (*Arachis hypogaea* L.)

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## Summary

A method of field screening groundnut seedlings for resistance to groundnut rosette virus (GRV), by means of which over 97% incidence was induced in rows of susceptible test plants, was developed at Chitedze Research Station in Malawi. Two GRV-resistant Virginia cultivars (RG 1 and RMP 40) were crossed with three susceptible cultivars, one from each of the Spanish (JL 24), Valencia (ICGM 48) and Virginia (Mani Pintar) botanical groups. Twelve $F_1$ reciprocal crosses and their $F_2$ and backcross generations were produced and the material screened in nurseries in 1985/86 and 1986/87. Seedlings raised from plants which did not become infected in the field were inoculated in the glasshouse in order to eliminate susceptible escapees.

The numbers of diseased and healthy individuals in each population were subjected to $\chi^2$ tests. In the majority of the $F_2$ populations a good fit was obtained for a ratio of one resistant to 15 susceptible plants, a ratio to be expected if resistance to GRV were determined by a pair of independent complementary recessive genes. This was further supported by data from backcross generations.

**Key words:** Groundnut, groundnut rosette virus, inheritance, complementary genes, field screening method

## Introduction

Cultivated groundnut, a native of South America, is an important oil and food crop in the world. It is cultivated on 19.53 m ha of which 5.72 m are grown in Africa (Anon., 1989). It has two subspecies which, in turn, have two botanical varieties each. They are subsp. *hypogaea* var. *hypogaea* (Virginia), subsp. *hypogaea* var. *hirsuta*, subsp. *fastigiata* var. *fastigiata* (Valencia), and subsp. *fastigiata* var. *vulgaris* (Spanish). Varieties belonging to subsp. *hypogaea* have procumbent, decumbent or erect growth habit; alternate branching; simple inflorescences which are never borne directly on the main axis; first branch on the cotyledonary laterals always vegetative; usually dormant seeds, and dark green foliage. In contrast, the varieties belonging to subsp. *fastigiata* have decumbent or erect growth habit; sequential branching; simple or compound inflorescences always present on main axis; first branch on the cotyledonary laterals always reproductive; usually non-dormant seeds, and light green foliage. They also mature earlier than subsp. *hypogaea* varieties.

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Cultivated groundnut, a self pollinator, is an allotetraploid (2 n = 40) with a basic chromosome of 10 (x = 10). However, it has disomic inheritance.

Groundnut rosette disease is the most damaging virus disease of groundnut, and occurs sporadically in severe epidemics, particularly in West Africa: in the 1975 epidemic in Northern Nigeria, an estimated 0.7 million ha were destroyed (Yaycock, Rossel & Harkness, 1976). It is apparently endemic to Africa, and is limited to the African continent and its off-shore islands. It is transmitted by *Aphis craccivora* (Storey & Bottomley, 1928) in the persistent manner.

Two main symptom types occur, described as chlorotic rosette (Storey & Bottomley, 1928) and green rosette (Hayes, 1932). Chlorotic rosette has been reported from most countries south of the Sahara. The distribution of green rosette is imperfectly known. It has been reported from West Africa and Uganda, but as yet there are no authenticated reports of its occurrence in Kenya, Tanzania or southern Africa.

Three agents are involved in rosette disease, groundnut rosette virus (GRV), groundnut rosette assistor virus (GRAV) and a satellite RNA (Murant, Rajeshwari, Robinson & Raschke, 1988). GRV is dependent upon GRAV for transmission by its aphid vector (Hull & Adams, 1968), and the satellite RNA (which is largely responsible for rosette symptoms in groundnut) is itself dependent on GRV for replication (Murant *et al.*, 1988).

Resistance to rosette in the cultivated groundnut was discovered in local landraces in Burkina Faso (De Berchoux, 1958). These landraces belonged to the Virginia variety group and were late maturing and poor yielders. De Berchoux (1960) later showed that this resistance was controlled by two independent recessive genes. He also observed that resistant lines were not immune and that individual plants could become infected with GRV when subjected to inoculation by massive numbers of aphids. This resistance apparently operates equally against both chlorotic (De Berchoux, 1960) and green (Harkness, 1977) rosette.

Recent studies (Murant, Bock & Rajeshwari, 1989 and unpublished data) have shown that this resistance is directed against GRV and thus brings with it resistance to the satellite RNA: the plants are fully susceptible to GRAV, which alone induces no obvious symptoms.

Harkness (1977) reported a low recovery of resistant plants from Virginia × Spanish crosses. He attributed this to heavy inoculation pressure at early stages of plant growth, and suggested loss of resistance from generation to generation might be expected if the recessive genes did not confer resistance in all nuclear backgrounds.

There have also been reports (Misari *et al.*, 1988) which suggest that rosette resistance may not be as simply inherited as first described. Because of these uncertainties, and because previous definitive work on inheritance of resistance was confined to Virginia × Virginia crosses ($F_1$ and $F_2$ generations) in West Africa, a detailed study was made of the inheritance of resistance in crosses between Virginia, Valencia and Spanish types. This paper reports the results of this study, which was conducted at Chitedze Agricultural Research Station, Malawi.

## Materials and Methods

Two GRV-resistant Virginia cultivars (RG 1 and RMP 40) were crossed with three susceptible cultivars, one from each of the Spanish (JL 24), Valencia (ICGM 48), and Virginia (Mani Pintar) botanical groups.

*RG1* was derived from the cross Makulu Red × 48-14, and is recommended for cultivation in rosette-prone areas in Malawi.

*RMP 40* is an original West African selection obtained from crosses of rosette-resistant lines × Mani Pintar.

*JL 24* was mass-selected for earliness and yield in India from germplasm line EC 94943, which was introduced from Taiwan in 1971. The line used in this study was re-selected in Malawi from an importation from ICRISAT, India.

*ICGM 48* originated in Brazil and is maintained in USDA collection as PI 152132.

*Mani Pintar*, a distinctive variety with a variegated testa, was collected in Bolivia. It was introduced into Zambia in 1955; pure red-seeded lines were selected from it and the best of these distributed as Makulu Red (see RG 1 above).

Twelve $F_1$ reciprocal crosses involving resistant and susceptible parents and their $F_2$ and backcross generations were produced and the material screened in rosette disease nurseries in the field in the 1985/86 and 1986/87 rainy seasons. The two resistant parents were also crossed with each other and the $F_1$ and $F_2$ generations were screened together with the other material.

The GRV isolate used in the experiments was obtained from a typical chlorotic rosette field infection at Chitedze in 1984, and was maintained in the glasshouse by standard serial passage using *A. craccivora*.

The management of rosette nurseries was based on the observed pattern of natural GRV spread in Malawi, where only primary infections give rise to typical patches of rosette disease, in which all or nearly all plants are infected. Such primary infections invariably occur subsequent to the earliest migrations of the vector, at or shortly after emergence of the crop.

At normal sowing time, generally at the onset of the rains, one infector row of the highly susceptible variety Malimba was planted after every two contiguous rows of test lines, such that every test row was adjacent to one infector row. Well in advance of this period, large numbers of susceptible seedlings (cv. Spancross) were raised in the glasshouse and inoculated with GRV, using viruliferous aphids which had been reared on GRV-infected plants. After 5-7 days the aphids were killed using pirimiphosmethyl. When symptoms of rosette had appeared, infected plants were re-infested with viruliferous aphids and dense populations of apterae were allowed to develop.

About one wk after emergence of the seedlings in the field nursery, the infected glasshouse plants, heavily infested with aphids, were transplanted at 1.5 m intervals into the infector rows. Subsequently, the nursery was randomly seeded on several occasions with viruliferous aphids from glasshouse cultures.

This technique resulted in a GRV incidence in infector rows of 99% and 98% in the 1985/86 and 1986/87 seasons, respectively, and 97% in rows of susceptible plants used as test lines to monitor the efficacy of the method in the 1986/87 season (Bock & Nigam, 1988).

Because of the apparent difficulty of achieving 100% incidence in field tests, the healthy survivors of test lines were further screened in the glasshouse. Three to five seeds from each survivor were raised and each seedling was inoculated with GRV on two or three occasions, at 14-day intervals, using batches of 10 viruliferous aphids per plant. If any one seedling was susceptible in this test, its progenitor was recorded as susceptible. This test helped in eliminating susceptible plants which had escaped infection in the field.

## Results and Discussion

Reactions of the five parents and their 12 $F_1$ crosses to GRV infection are given in Table 1. In both seasons, all plants of the two resistant parents, RG 1 and RMP 40, remained free of infection, and all plants of one susceptible parent, JL 24, were diseased. While all plants of both other susceptible parents (ICGM 48 and Mani Pintar) were diseased in the 1986/87

Table 1. *Reaction of parents and their $F_1$ generation crosses to infection by groundnut rosette virus*

| Genotype | Number of healthy (H) and diseased (D) plants 1985/86 H:D | 1986/87 H:D | Total H:D |
|---|---|---|---|
| *Resistant parents* | | | |
| RG 1 | 85:0 | 10:0 | 95:0 |
| RMP 40 | 93:0 | 10:0 | 103:0 |
| *Susceptible parents* | | | |
| JL 24 | 0:35 | 0:20 | 0:55 |
| ICGM 48 | 4:51 | 0:15 | 4:66 |
| Mani Pintar | 3:50 | 0:19 | 3:69 |
| *$F_1$ crosses* | | | |
| (RG 1 × JL 24) | 1:3 | 0:5 | 1:8 |
| (JL 24 × RG 1) | 0:6 | 0:5 | 0:11 |
| (RG 1 × ICGM 48) | 0:5 | 2:2 | 2:7 |
| (ICGM 48 × RG 1) | 0:5 | 0:5 | 0:10 |
| (RG 1 × Mani Pintar) | 0:5 | 0:5 | 0:10 |
| (Mani Pintar × RG 1) | 0:6 | 0:5 | 0:11 |
| (RMP 40 × JL 24) | 0:4 | 0:5 | 0:9 |
| (JL 24 × RMP 40) | 0:4 | 0:5 | 0:9 |
| (RMP 40 × ICGM 48) | 0:5 | 0:5 | 0:10 |
| (ICGM 48 × RMP 40) | 0:5 | 0:5 | 0:10 |
| (RMP 40 × Mani Pintar) | 1:4 | 1:4 | 2:8 |
| (Mani Pintar × RMP 40) | 0:4 | 0:5 | 0:9 |

tests, a few plants did not develop GRV symptoms in the 1985/86 tests. During routine screening in the rosette resistant cultivar development programme, this anomalous behaviour was encountered, infrequently, in other susceptible cultivars, when individual plants did not develop disease symptoms even after repeated inoculations. However, when these plants were progeny-rowed, all their progenies were found to be susceptible.

Most $F_1$ crosses were uniformly diseased except for a few plants in the RG 1 × JL 24, RG 1 × ICGM 48, and RMP 40 × Mani Pintar crosses. These plants could have arisen from selfed seed, as in all three crosses the female parent was the resistant parent.

The $F_2$ data on disease reaction were subjected to $\chi^2$ tests for a predicted ratio of 1 resistant: 15 susceptible plants (Table 2). All six crosses involving RG 1 as the parent showed a good fit for this ratio in both seasons. Four of the six RMP 40 crosses in the 1985/86 season and three of the four in the 1986/87 season also showed a good fit for this ratio. In all three cases where a good fit was not obtained, there was an excess of diseased plants. Two such cases were of the JL 24 (Spanish) × RMP 40 (Virginia) cross. Similarly, Harkness (1977) reported a low recovery of resistant plants from Virginia × Spanish crosses under heavy inoculation pressure at early stages of plant growth.

Data on backcrosses with resistant parents were subjected to $\chi^2$ tests for a predicted ratio of 1 resistant: 3 susceptible plants (Table 3). Except for the (RMP 40 × JL 24) × RMP 40 cross in the 1986/87 season, all other backcrosses with both resistant parents gave a good fit for this ratio. In the (RMP 40 × JL 24) × RMP 40 cross, there was an excess of healthy plants. This could have been due to mis-identification of RMP 40 selfed plants as genuine $F_1$ plants while making the backcrosses in the field.

Table 2. *Chi-square test for a 1:15 ratio of plants segregating for resistance to groundnut rosette virus in the $F_2$ generation*

| Cross | Number of healthy (H) and diseased (D) plants (1985/86) | $\chi^2$ | Number of healthy (H) and diseased (D) plants (1986/87) | $\chi^2$ |
|---|---|---|---|---|
| | H:D | | H:D | |
| (RG 1 × JL 24) | 35:596 | 0.5325 NS | 51:891 | 1.1236 NS |
| (JL 24 × RG 1) | 29:579 | 2.2737 NS | 44:848 | 2.6415 NS |
| Pooled | 64:1175 | 2.4872 NS | 95:1739 | 3.5840 NS |
| (RG 1 × ICGM 48) | 73:1012 | 0.4233 NS | 5:160 | 2.9192 NS |
| (ICGM 48 × RG 1) | 12:160 | 0.1550 NS | — | — |
| Pooled | 85:1172 | 0.5627 NS | — | — |
| (RG 1 × Mani Pintar) | 86:1195 | 0.4697 NS | 4:73 | 0.1462 NS |
| (Mani Pinta × RG 1) | 35:564 | 0.5317 NS | 63:1109 | 1.5299 NS |
| Pooled | 119:1759 | 0.0240 NS | 67:1182 | 1.6722 NS |
| Pooled over all RG 1 crosses | 268:4106 | 0.1127 NS | 167:3081 | 6.8098** |
| Heterogeneity | | 4.2732 NS | | 1.5506 NS |
| (RMP 40 × ICGM 48) | 39:856 | 5.4705* | 57:965 | 0.7893 NS |
| (ICGM 48 × RMP 40) | 7:145 | 0.7017 NS | 9:69 | 3.7231 NS |
| Pooled | 46:1001 | 6.1586* | 66:1034 | 0.1075 NS |
| (RMP 40 × Mani Pintar) | 19:442 | 3.5646 NS | — | — |
| (Mani Pintar × RMP 40) | 57:663 | 3.4133 NS | — | — |
| Pooled | 76:1105 | 0.0691 NS | — | — |
| (RMP 40 × JL 24) | 25:437 | 0.5547 NS | 17:325 | 0.9552 NS |
| (JL 24 × RMP 40) | 24:617 | 6.8694** | 14:429 | 7.2176** |
| Pooled | 49:1054 | 6.1506* | 31:754 | 7.0931** |
| Pooled over all RMP 40 crosses | 171:3160 | 7.0854** | 97:1788 | 3.9218* |
| Heterogeneity | | 13.4888* | | 8.7634* |

NS: Non-significant at 0.05 level of significance.
*, **: Significant at 0.05 and 0.01 level of significance, respectively.

The majority of plants in backcrosses to susceptible parents were diseased (Table 4). Only three plants in two crosses of RG 1 and 19 plants in five crosses of RMP 40, over both seasons did not show disease symptoms.

Data derived from $F_2$ and backcross generations generally support De Berchoux's (1960) conclusion that resistance to GRV in groundnut is governed by a pair of independent complementary recessive genes. With RG 1 as the resistant parent, segregation patterns in the $F_2$ and backcross generations of the resistant parent are in complete agreement with the hypothesis.

When the resistant parents were crossed with each other, all $F_1$ plants were healthy and, in the $F_2$ generation, only eight plants out of 3207 were diseased (Table 5). As both RG 1 and RMP 40 derive their resistance from the same original resistant landrace sources, the occurrence of these susceptible plants may possibly be due to a low level of outcrossing in groundnut (Gibbons & Tattersfield, 1969).

Table 3. *Chi-square test for a 1:3 ratio of plants segregating for resistance to groundnut rosette virus in backcrosses with resistant parents*

| Cross | Number of healthy (H) and diseased (D) plants (1985/86) | $\chi^2$ | Number of healthy (H) and diseased (D) plants (1986/87) | $\chi^2$ |
|---|---|---|---|---|
| | H:D | | H:D | |
| (RG 1 × JL 24) × RG 1 | 25:104 | 2.1731 NS | 4:22 | 1.2820 NS |
| (JL 24 × RG 1) × RG 1 | 22:83 | 0.9174 NS | 9:32 | 0.2032 NS |
| Pooled | 47:187 | 3.0142 NS | 13:54 | 1.1194 NS |
| (RG 1 × ICGM 48) × RG 1 | 43:128 | 0.0019 NS | 7:17 | 0.2222 NS |
| (ICGM 48 × RG 1) × RG 1 | 9:18 | 1.0000 NS | 1:4 | 0.0667 NS |
| Pooled | 52:146 | 0.1683 NS | 8:21 | 0.1034 NS |
| (RG 1 × Mani Pintar) × RG 1 | 42:160 | 1.9076 NS | 9:35 | 0.4848 NS |
| (Mani Pintar × RG 1) × RG 1 | 19:83 | 2.2091 NS | 6:15 | 0.1428 NS |
| Pooled | 61:243 | 3.9473* | 15:50 | 0.1282 NS |
| Pooled over 6 crosses | 160:576 | 4.1739* | 36:125 | 0.5983 NS |
| Heterogeneity | | 4.0352 NS | | 1.8034 NS |
| (RMP 40 × JL 24) × RMP 40 | 41:120 | 0.0186 NS | 11:15 | 4.1538* |
| (JL 24 × RMP 40) × RMP 40 | 21:38 | 3.5311 NS | 5:26 | 1.3011 NS |
| Pooled | 62:158 | 1.1879 NS | 16:41 | 0.2865 NS |
| (RMP 40 × ICGM 48) × RMP 40 | 12:21 | 2.2727 NS | 4:6 | 1.2000 NS |
| (ICGM 48 × RMP 40) × RMP 40 | 11:54 | 2.2615 NS | 1:3 | 0.0000 NS |
| Pooled | 23:75 | 0.1224 NS | 5:9 | 0.8571 NS |
| (RMP 40 × Mani Pintar) × RMP 40 | 33:117 | 0.7200 NS | 13:28 | 0.9837 NS |
| (Mani Pintar × RMP 40) × RMP 40 | 27:72 | 0.2727 NS | 4:14 | 0.0741 NS |
| Pooled | 60:189 | 0.1084 NS | 17:42 | 0.4576 NS |
| Pooled over 6 crosses | 145:422 | 0.0993 NS | 38:92 | 1.2410 NS |
| Heterogeneity | | 8.9773 NS | | 6.4717 NS |

NS: Non-significant at 0.05 level of significance.
*: Significant at 0.05 level of significance.

Table 4. *Reaction of plants to infection of groundnut rosette virus in backcrosses with susceptible parents*

| Cross | Number of healthy (H) and diseased (D) plants | | |
|---|---|---|---|
| | 1985/86 | 1986/87 | Total |
| | H:D | H:D | H:D |
| (RG 1 × JL 24) × JL 24 | 0:159 | 0:60 | 0:219 |
| (JL 24 × RG 1) × JL 24 | 0:92 | 0:52 | 0:144 |
| (RG 1 × ICGM 48) × ICGM 48 | 1:119 | 1:24 | 2:143 |
| (ICGM 48 × RG 1) × ICGM 48 | — | 0:2 | 0:2 |
| (RG 1 × Mani Pintar) × Mani Pintar | 0:197 | 1:27 | 1:224 |
| (Mani Pintar × RG 1) × Mani Pintar | 0:86 | 0:23 | 0:109 |
| (RMP 40 × JL 24) × JL 24 | 0:84 | 0:46 | 0:130 |
| (JL 24 × RMP 40) × JL 24 | 0:116 | 1:35 | 1:151 |
| (RMP 40 × ICGM 48) × ICGM 48 | 2:142 | 3:63 | 5:205 |
| (ICGM 48 × RMP 40) × ICGM 48 | 3:62 | 3:13 | 6:75 |
| (RMP 40 × Mani Pintar) × Mani Pintar | 5:164 | 0:19 | 5:183 |
| (Mani Pintar × RMP 40) × Mani Pintar | 2:126 | 0:52 | 2:178 |

Table 5. *Reaction of plants to infection of groundnut rosette virus in the $F_1$ and $F_2$ generations of crosses of resistant parents*

| Cross | Number of healthy (H) and diseased (D) plants | | |
|---|---|---|---|
| | 1985/86 | 1986/87 | Total |
| | H:D | H:D | H:D |
| *$F_1$ cross* | | | |
| (RG 1 × RMP 40) | 4:0 | 5:0 | 9:0 |
| (RMP 40 × RG 1) | 5:0 | 5:0 | 10:0 |
| *$F_2$ cross* | | | |
| (RG 1 × RMP 40) | 1838:6 | 83:0 | 1921:6 |
| (RMP 40 × RG 1) | 544:0 | 734:2 | 1278:2 |

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