Mechanical Inoculation Procedure to Screen for Resistance to Groundnut Rosette Virus in Peanut[1]

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ABSTRACT

A mechanical inoculation procedure was developed for screening peanut plants for resistance to groundnut rosette virus (GRV) (green strain). A single inoculation resulted in 100% infection of plants of susceptible genotypes and about 2% of resistant plants which could be distinguished from susceptible ones on the basis of delayed time of first appearance of symptoms and disease severity. The procedure utilized highly infectious inoculum. The consistency and reliability of achieving infection should make the procedure particularly effective for inheritance of resistance studies and breeding programs.

Key Words: Virus transmission, screening for resistance, virus inoculation, groundnut rosette virus.

Two viruses, groundnut rosette virus (GRV) and groundnut rosette assistor virus (GRAV), are associated with the groundnut rosette disease. In peanut (*Arachis hypogaea* L.), GRV induces symptoms which are associated with the production of a satellite RNA [900 base pairs, double-stranded (ds)RNA] (Breyel *et al.*, 1988; Murant *et al.*, 1988), whereas GRAV alone causes no symptoms but is essential for aphid transmission of GRV. GRV can be transmitted mechanically but GRAV, a luteovirus, is not mechanically transmitted (Okusanya and Watson, 1966).

In general, mechanical inoculation of GRV to peanut has been difficult to achieve. Although research has been conducted on groundnut rosette since 1907 (Zimmerman, 1907), there were no reports of mechanical transmission prior to 1966. Results since then have been inconsistent and 100% infection has rarely been reported. Okusanya and Watson (1966) obtained one of 10 and five of 12 infected plants by mechanical inoculation; Hull and Adams (1968) reported 50-100% infection; Rossel (1977) obtained 0 of 200 infected plants; Reddy *et al.* (1985) reported 34 of 48 plants infected; and Murant *et al.* (1988) obtained 17 of 18 and 11 of 18 infected plants in different tests. Thus, it is easy to understand why general resistance studies (deBerchoux, 1958; Nutman *et al.*, 1964) and inheritance of resistance studies (deBerchoux, 1960; Harkness, 1977; Nigam and Bock, 1990) have been conducted with graft or aphid inoculation of mixed infections of GRV and GRAV.

The objective of this study was to develop a consistent and reliable procedure to screen peanut for resistance to single infections of GRV. A preliminary report has been made (Olorunju *et al.*, 1990).

Materials and Methods

The inoculation buffer contained 0.1 M potassium phosphate (pH 7.4), 0.2% mercaptoethanol, (Hull and Adams, 1968; Reddy *et al.*, 1985), and 1% magnesium bentonite which had been prepared according to Dunn and Hitchborn (1965). The original virus inoculum was obtained from field-grown peanut with green rosette near Samaru, Nigeria. The identity of the causal agents was confirmed by diagnostic tests for the GRV dsRNA (Breyel *et al.*, 1988) and serological relatedness to a luteovirus antiserum (Casper *et al.*, 1983). After several transfers from peanut to peanut by aphid transmission, a single infection of GRV was established by mechanical inoculation to peanut.

Test peanut genotypes included GRV-susceptible F 452.4 and GRV-resistant RMP 12 and RG 1. Resistant F_2s, F_3s, and F_4s were selected resistant plants from crosses (resistant x susceptible) of selfed generations of F_2, F_3, and F_4

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(Olorunju *et al.*, 1991, 1992). A total of 107 susceptible and 473 resistant plants were tested for rosette disease reaction.

Plants were grown in 10-cm diameter pots and maintained in a glasshouse where weekly applications of the insecticide dichlorvos (Nuvan) were made to control aphids. Prior to inoculation, 5- to 7-d old seedlings were kept in the dark for 24 hr. They were removed from the dark 1 hr before inoculation to allow closed leaflets to open. Four young leaflets from F 452.4 plants showing distinct mottle (7 to 12 d after mechanical inoculation) were ground in a cold mortar with 6.0 mL of buffer (approximately a 1/30 dilution). Both the upper and lower leaf surfaces were dusted with carborundum and rubbed with inoculum, using latex gloves (to protect from ribonuclease) and a cheesecloth pad. Fresh inoculum was prepared every 5 to 8 min (about 25 plants inoculated). In order to remove inoculum debris and prevent desiccation from inoculation injury, inoculated plants were sprayed with an atomizer three to four times, at 10-min intervals, with distilled water. In the tests during May and July the glasshouse temperatures averaged 22 C low and 36 C high with day lengths greater than 12 hr. In the tests during October and December the glasshouse temperatures averaged 20 C low and 32 C high with day lengths less than 12 hr. Inoculated plants were observed daily for first symptom appearance, which was recorded for each plant.

Disease severity at 90 d after inoculation was evaluated according to the following categories of symptoms: 1 = none, 2 = discernible rosette leaf symptoms (no stunt), 3 = rosette leaf symptoms plus stunt ranging from barely discernible to about 15%, 4 = rosette leaf symptoms plus stunt ranging from about 15-50%, and 5 = rosette leaf symptoms plus stunt greater than 50%. Severity indices were determined in a manner similar to that described by Kuhn and Smith (1977) as follows: (A+2B+3C+4D+5E) ÷ number of plants per treatment where A, B, C, D, and E equals the number of plants with ratings of 1, 2, 3, 4, and 5, respectively. For confirmation of virus diagnosis and symptoms, the simple extraction and electrophoresis procedure described by Breyel *et al.* (1988) was used to detect the 900 base pair dsRNA associated with GRV infections.

Results

The initial mechanical transmission from aphid-inoculated plants to F 452.4 usually resulted in 10 to 50% incidence of infection. By the third or fourth mechanical transmission from mechanically inoculated plants that developed symptoms, the infection incidence increased to 100% and remained at that level as long as young leaflets from plants infected 7 to 12 d was used as inoculum.

Plants of cultivar F 452.4 in each of four tests developed GRV symptoms within 16 d after a single inoculation (Table 1). Symptoms appeared as early as 4 d after inoculation and most plants had symptoms by 8 d. In four tests, only 2% of the resistant plants developed symptoms (Table 1). Resistant plants could be distinguished from susceptible ones on the basis of time of symptom appearance (Table 1) and disease severity (Table 2).

In susceptible plants, warm to hot temperatures in May and July apparently caused GRV symptoms to appear earlier and to be more severe than cooler temperatures in October and December (Tables 1 and 2). In the December test, none of 368 resistant plants became diseased.

Multiple inoculations of resistant plants with GRV at 1- or 2-d intervals, or after all susceptible plants showed symptoms, caused additional plants to become diseased but 100% infection was not observed. In Test 1, for example, 22 of the 33 resistant plants eventually (45 d after first inoculation) developed symptoms following four inoculations (Table 1).

Electrophoresis diagnostic tests established that the GRV 900 bp dsRNA was present in both susceptible and resistant plants with symptoms. However, not all of the resistant plants with mild symptoms were positive, pre-

Table 1. Disease incidence following mechanical inoculation of peanut genotypes with groundnut rosette virus (green strain) in the greenhouse.

Test No.	Test Date	Type of genotype[a]	New plants diseased after inoculation (d) 4	8	12	16	Plants diseased/ no. plants inoculated
			---- no. ----				
1[b]	May	Resistant	0	0	5	2	7/33
		Susceptible	1	14	2	0	17/17
2	July	Resistant (F_2)	0	0	3	0	3/48
		Susceptible	1	20	8	0	29/29
3	Oct.	Resistant	0	0	1	0	1/24
		Susceptible	0	6	7	0	13/13
4	Dec.	Resistant (F_3, F_4)	0	0	0	0	0/368
		Susceptible	0	6	30	12	48/48

[a]In Tests 1 and 3 the resistant plants are RMP-12 or RG-1; the susceptible in all tests is F452.4; the resistant F_2s, F_3s, and F_4s are selected resistant plants from crosses (resistant x susceptible) of selfed generations of F_2, F_3, and F_4.

[b]In Test 1 only, resistant plants were inoculated four times.

Table 2. Symptom category and severity indices of peanut plants mechanically inoculated with groundnut rosette virus (green strain) in the greenhouse.

Test no.	Type of genotype[a]	Plants/disease category[b] Resistant 1	2	Susceptible 3	4	5	Severity indices[c]
1	Resistant	26	6	0	1	0	1.3
	Susceptible	0	0	0	0	17	5.0
2	Resistant (F_2)	42	4	2	0	0	1.2
	Susceptible	0	0	0	8	21	4.7
3	Resistant	23	0	1	0	0	1.1
	Susceptible	0	0	0	9	4	4.3
4[d]	Resistant (F_3, F_4)	368	0	0	0	0	1.0
	Susceptible	0	0	12	36	0	3.8

[a]In Tests 1 and 3 the resistant plants are RMP-12 or RG-1; the susceptible in all tests is F452.4; the resistant F_2s, F_3s, and F_4s are selected resistant plants from crosses (resistant x susceptible) of selfed generations of F_2, F_3, and F_4.

[b]Symptom categories: 1 = no symptoms, 2 = discernible rosette leaf symptoms (no stunt), 3 = rosette leaf symptoms plus stunt ranging from barely discernible to about 15%, 4 = rosette leaf symptoms plus stunt ranging from 15-50%, 5 = rosette leaf symptoms plus stunt greater than 50%.

[c]Severity indices were determined as follows: (A+2B+3C+4D+5E) ÷ number of plants per treatment where A, B, C, D, and E equals the number of plants with symptom categories of 1, 2, 3, 4, and 5, respectively.

[d]In Test 4, disease ratings were taken 30 d after inoculation instead of 90 d as for Tests 1, 2, and 3.

sumably because of a relationship between disease severity and concentration of the dsRNA (Olorunju, 1990).

Discussion

Field screening procedures to evaluate segregating populations for their reaction to GRV and GRAV are difficult to manage. In an inheritance study, Olorunju *et al.* (1992) followed a procedure developed by Nigam and Bock (1990) which was similar to one used previously by Harkness (1977). The procedure is laborious and time consuming. It requires transplanting large numbers of infected plants into the field, establishment and release of viruliferous aphids one or more times, timing of events that are dependent on weather conditions, and introduction of virus inoculum into relatively large experimental plots in a geographic area which might be detrimental to commercial production. Olorunju *et al.* (1991, 1992) reported 11% of susceptible plants did not develop symptoms in 1988 whereas 87% of resistant plants developed symptoms in 1989. Such variability in results make disease classification of plants difficult in studies to measure the inheritance of resistance. Achieving a balance between too high and too low inoculum pressure is risky and would be variable from year to year.

This mechanical inoculation study was the first attempt to screen for resistance to a single infection by GRV. Segregation patterns in F_2 and F_3 populations were similar to and less equivocal than those in field studies with mixed infections of GRV and GRAV (Olorunju, 1990, 1992). Mechanical inoculation was very effective in separating resistant and susceptible plants. The probability of susceptible escapes proved to be very low and the method allowed for reinoculation of symptomless plants.

We recommend screening of peanut germplasm by mechanical inoculation with GRV for both genetic studies and subsequent backcross and selection in later generations. The process is time (results in 3 to 4 wk) and labor efficient and accommodates mating designs that do not limit the number of parents, thus providing allowance for genetic diversity and maximum genetic information which would otherwise be restricted due to population size. The potential complication of mixed virus infection can be avoided. Greenhouse screening neither interferes with field operations, as it could be done during the noncropping season, nor with conventional breeding methods, but it helps in advancing the segregating generations faster to the F_5 generation where selection for other traits such as yield begins. Highly infectious inoculum in the greenhouse can be relatively controlled and the inoculum can be applied multiple times, thus increasing confidence in detection of plants homozygous for resistance at an early generation. Such plants can be easily transplanted to the field for further observation under severe disease conditions (mixed infections of GRV and GRAV) and for seed production for the next generation.

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