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AFLP Diversity Among Selected Rosette Resistant Groundnut Germplasm

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Groundnut rosette is the most destructive disease of groundnut (*Arachis hypogaea*) in Africa. It is sporadic and unpredictable but causes significant loss in groundnut in years of epidemics (Naidu et al. 1999). Three synergistic agents cause rosette disease: groundnut rosette virus (GRV), a satellite RNA of GRV and groundnut rosette assistor virus (GRAV) (Bock et al. 1990). All three agents need to be present in the plants for aphid (*Aphis craccivora*) transmission. Resistance to groundnut rosette has been detected in 116 accessions of *A. hypogaea*. These accessions possess resistance to GRV but are susceptible to GRAV (Subrahmanyam et al. 1998). A few rosette resistant accessions are also resistant to *Aphis craccivora* (Padagham et al. 1990, Minja et al. 1999). These represent a wide range of biotypes and landraces from Latin America, Africa, and Asia, but their genetic relationships are not known.

Molecular marker-based diversity estimates are useful to select diverse lines for developing populations that may be used for mapping studies to identify DNA markers linked with resistance to rosette in groundnut. Nine amplified fragment length polymorphism (AFLP) assays (Vos et al. 1995), using primer pairs E-ACA + M-CAA, E-ACA + M-CAG, E-AGC + M-CTG, E-AGC + M-CTA, E-ACT + M-CAG, E-ACC + M-CAG, E-ACC + M-CAA, E-AAC + M-CTG and E-AAC + M-CAG, were performed on nine rosette resistant (ICGs 3436, 6323, 6466, 9558, 9723, 10347, 11044, 11968 and 12876) and one susceptible (ICG 7827) groundnut accessions. Young leaves from 2-week old plants were bulk harvested for each accession and immediately placed in liquid nitrogen for DNA extraction. DNA was extracted using the CTAB method (Saghai-Maroof et al. 1984). The concentration of DNA was assessed by spectrophotometer analyses, and the quality by gel electrophoresis using 0.8% agarose with a known concentration of uncut lambda DNA. 500 ng of genomic DNA was double digested with *Eco*R 1 and *Mse* 1 in a restriction buffer in a total volume of 15 µl. *Mse* 1 and *Eco*R 1 adapters were subsequently ligated to digested

DNA fragments. The adapter-ligated DNA was pre-amplified using the following cycling parameters: 20 cycles of 30 s at 94°C, 60 s at 56°C and 60 s at 72°C. The pre-amplified DNA was diluted in a ratio of 1:50 prior to labeling it with $\gamma^{33}P$ that was used as template for the selective amplification with *Eco*R 1 and *Mse* 1 primers having three selective nucleotides at their 3′ end. The cycling parameter for selective amplification was 1 cycle of 30 s at 94°C, 30 s at 65°C, and 60 s at 72°C. The annealing temperature was lowered by 0.7°C $cycle^{-1}$ during the first 12 cycles, and then 23 cycles were performed at 94°C for 30 s, 56°C for 30 s and 70°C for 60 s. After the selective amplification, the reaction was stopped by the addition of 20 µl of formamide dye. The amplification product was separated by denaturing 6% polyacrylamide gel electrophoresis, and autoradiographs were manually scored as 1 for the presence and 0 for the absence of band from higher to lower molecular weight products.

Pair-wise genetic similarity (S_{ij}) between accessions i and j was estimated using the similarity coefficient of Nei and Li (1979) as $S_{ij} = 2 N_{ij} / (N_i + N_j)$, where N_{ij} is the number of bands common in accessions i and j, and N_i and N_j are the total numbers of bands in accessions i and j, respectively. S_{ij} represents the proportion of bands in common between any two accessions and may range from 0 (no common bands) to 1 (identical band profile for the two accessions). S_{ij} values were used to estimate genetic dissimilarity, as $D_{ij} = 1-S_{ij}$, and D_{ij} values were later on used to determine the relationships among lines using principal coordinate analysis (PCoA) (Sneath and Sokal 1973). All computations were performed using statistical computing package Genstat5 Release 4.1. A band was identified as a unique AFLP molecular marker if present in one line at a specific molecular weight but absent in the remaining lines for a given primer pair.

Across the 10 accessions the 9 primer pairs identified 94 unique markers, with an average of 10.4 markers per primer pair. The number of unique markers ranged from 1 for ICG 10347 and ICG 11968 to 49 for ICG 11044. Primer pair E-ACC + M-CAA detected 26 of the 32 unique markers present only in ICG 11044. Other primer pairs that detected high frequency of unique markers are E-AAC + M-CAG with 17 markers in ICG 6466 and E-ACC + M-CAG with 10 markers in ICG 6323. These unique AFLP markers could differentiate only 7 of the 10 accessions included in this study (Table 1). Accession specific markers were not detected in ICGs 9558, 9723, and 12876.

The genetic dissimilarity (D_{ij}) values ranged from 3.92% to 50.53% with an average of 19.56%. The D_{ij} matrix was used to determine the genetic relationships among lines using principal coordinate analysis (PCoA). Accession ICG 11044 (quadrant IV) and ICG 6323 and ICG 6466 (quadrant I) were well separated from each other as well as from the rest of the lines (Fig. 1). ICG 11044 with ICG 3436, ICG 9558 and ICG 11968 showed greater genetic diversity (36.59% to 50.53%) amongst the rosette resistant accessions. The former is a landrace from China whereas the latter three are landraces from Africa. They all belong to subsp *hypogaea* var *hypogae*a, and possess high levels of resistance to rosette, average ≤2% compared to ≥90% in susceptible control ICG 7827 (JL 24) across four seasons in evaluation at Lilongwe, Malawi. These accessions therefore may be inter-crossed among themselves to produce diversified rosette resistant breeding populations. ICG 3436, ICG 6323 and ICG 11044 also showed greater diversity (26.50% to 41.52%) with the susceptible accession ICG 7827. ICG 11044 (rosette resistant) and ICG 7827 (rosette susceptible) should be crossed for developing appropriate mapping

Table 1. Unique AFLP markers identified in 7 of the 10 groundnut accessions tested.

Primer pair	ICG 11044	ICG 10347	ICG 11968	ICG 7827	ICG 6323	ICG 3436	ICG 6466	Total
E-ACA + M-CAA	7	1						8
E-ACA + M-CAG	7							7
E-AGC + M-CTG	6							6
E-AGC + M-CTA			1					1
E-ACT + M-CAG				3				3
E-ACC + M-CAG				5	10			15
E-ACC + M-CAA	26			1		3	2	32
E-AAC + M-CTG	2							2
E-AAC + M-CAG	1				1	1	17	20
Total	49	1	1	9	11	4	19	94

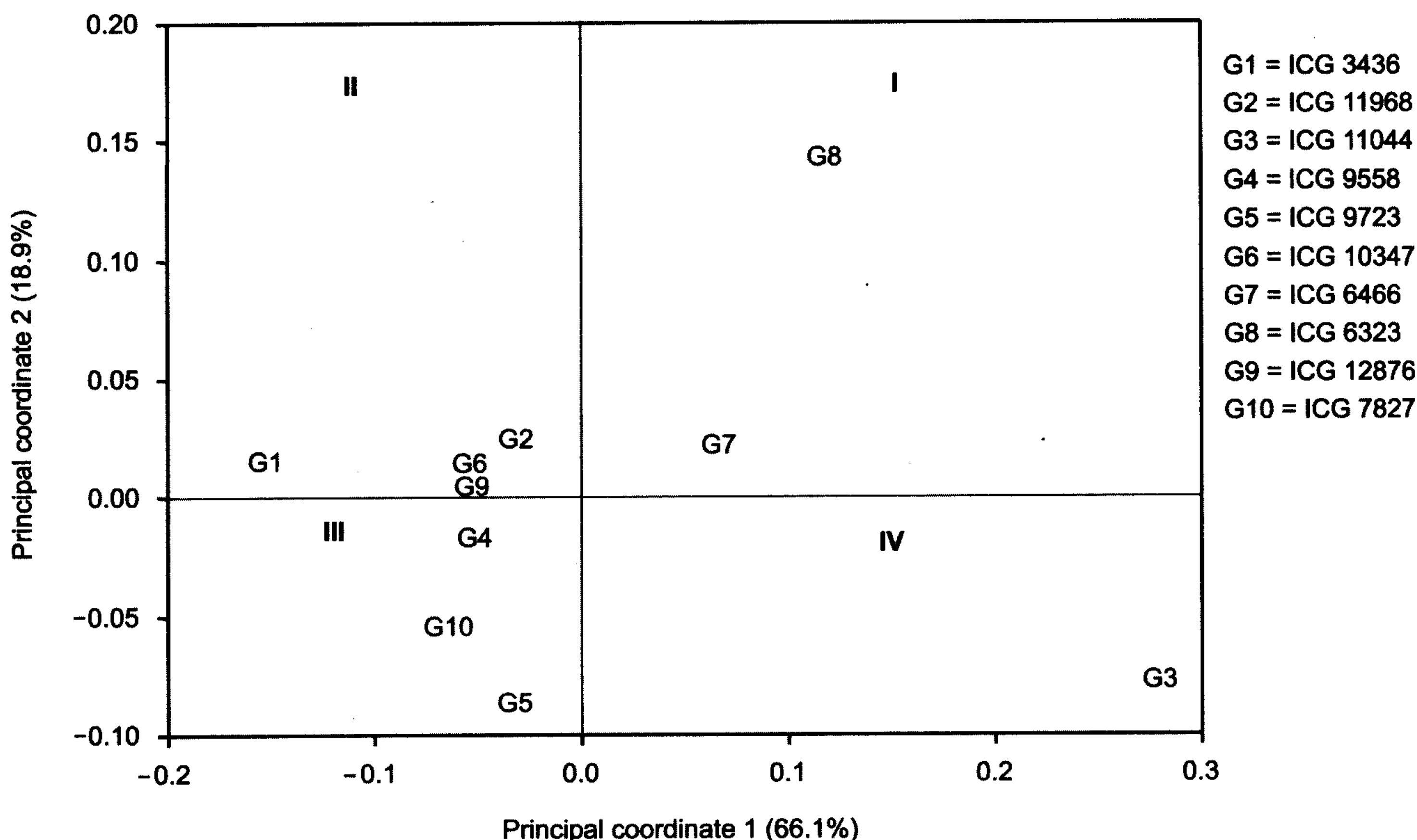

Figure 1. Relationships between 10 groundnut accessions as determined by principal coordinate analysis (PCoA) using AFLP-based dissimilarity matrix.

population (F_2 derived recombinant inbred lines) as their AFLP profiles differ by 41.52% and the former possess 49 unique AFLP markers that are absent in ICG 7827. The suggested AFLP primer pairs to identify markers linked with resistance to rosette in ICG 11044 × ICG 7827 are E-ACC + M-CAA, E-ACA + M-CAA, E-ACA + M-CAG and E-AGC + M-CTG as these showed maximum number of unique AFLP markers in ICG 11044.

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