



Recovery of Valencia Groundnut (*Arachis hypogaea* L.) Traits in Early Segregating and Promising Late Leaf Spot Resistant Populations

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Abstract. In Uganda, there are no Valencia varieties that are resistant to leaf spot diseases. Introgression of resistance genes into elite lines is always associated with the transfer of undesirable traits from the donor parents. Knowledge of the degree of genetic relationship provides breeders with a more efficient way to identify populations of potential relevance for their plant improvement programs. The objective of this study was to determine the recovery of Valencia groundnut traits in the early segregating and promising late leaf spot (LLS) resistant populations. Four crosses between Valencia lines (P1) and donor lines (P2) were made. The populations that included F1, F2, BC1P1 and BC1P2, together with their parents (P1 and P2) of each of the four crosses namely, Valencia C (P1) × ICGV-SM 02501 (P2), Valencia C (P1) × SGV-07009 (P2), NuMex-M3 (P1) × ICGV-SM 02501 (P2) and Redbeauty (P1) × ICGV-SM 03590 (P2) were evaluated. Cluster analysis revealed differences among the generations for the traits analysed with major and sub-clusters, implying that the generations of crosses formed a genetically diverse population that offers possible opportunity for selection. In the cross between NuMex-M3 X ICGV-SM 02501, the populations BC1P1 and F2, exhibited higher (80 %) recovery of the recurrent parental (NuMex-M3) traits. Similarly, the backcross population (BC1P1) to the susceptible elite variety of the Valencia C X ICGV-SM 02501 cross recovered approximately 75% of the recurrent parental traits Valencia C with lower LLS disease score. Such populations could result in higher gain in Valencia traits and LLS resistance when utilized the breeding program. For maximum gain in LLS resistance and recovery of Valencia traits, more selfing and backcrossing can be performed to fix the genes for LLS resistance as well those of Valencia characteristics.

Keywords: cluster analysis, *Arachis hypogaea*, diverse populations.

INTRODUCTION

The cultivated groundnut (*Arachis hypogaea*) is an annual herb with two subspecies. The subspecies *hypogaea* have been characterized by absence of flower on the main stem and alternate vegetative and reproductive nodes. It includes two botanical varieties *hypogaea* (*Virginia bunch* and *Virginia runner* types) and the less-frequently cultivated

hirsuta. The *fastigiata* subspecies is typified by flowers on the main stem and sequential reproductive nodes. It has four botanical varieties, *fastigiata* (Valencia type), *vulgaris* (Spanish type), *peruviana*, and *aequatoriana* (Krapovickas and Gregory, 1994).

In Uganda, there are no Valencia varieties that are resistant to leaf spot diseases (Okello *et al.*, 2010). The Valencia varieties are known for their highest score

for sweet taste and having three to five seeds per pod when compared with other botanical types (Patte *et al.*, 2001) valued by farmers because of earlier maturity and more convenient to cultivate. Traders also prefer Valencia due to higher oil content (Kaaya and Warren, 2005).

To broaden Valencia genetic base in Uganda, new high yielding breeding Valencia lines (Valencia C and NewMex-M₃) were introduced from New Mexico state USA and evaluated for resistance and tolerance to abiotic (drought) and biotic stresses (leaf spot, rosette and rust diseases and pests) at National Semi-Arid Resources Research Institute (NaSARRI) in Uganda. However, these cultivars were found susceptible to leaf spot diseases (Okello *et al.*, 2010). Sources of resistance to LLS were identified in ICGV-SM 03590 and ICGV-SM 02501 from ICRISAT Malawi, and SGV AL (advanced breeding line) lines which are Virginia and Spanish types (Okello *et al.*, 2010). The use of these lines in the introgression of high-level leaf spot resistance into agronomically desirable and high yielding groundnut varieties will be the most effective and economical management option under resource-limited farming systems in developing countries. However, introgression of resistance genes into elite lines is always associated with the transfer of undesirable traits from the donor parents. The objective of this study was to determine the morphological relationship among Valencia parental lines and their derived early segregating and promising late leaf spot (LLS) resistant populations. Knowledge on the degree of genetic relationship will help breeders in designing more efficient and effective breeding programs which will enhance the development and utilization of desirable LLS resistant cultivars with acceptable Valencia traits.

METHODS

The research was conducted at the National Semi-Arid Resources Research

Institute (NaSARRI) of the National Agricultural Research Organization (NARO) located 01° 30' 00"N and 33° 33' 00"E in Serere district, Uganda. This location represents a humid and hot climate that receives an annual rainfall 1,000 – 1,200 mm.

Materials

In the study seven groundnut genotypes (Table 1), with varying levels of response to LLS were used. The genotype had been characterized for resistance to LLS by the Groundnut Improvement Programme at NaSARRI. Four crosses between Valencia (P1) and donor (P2) lines were made. The six generations namely P₁, P₂, F₁, F₂ and BC₁P₁ and BC₁P₂ of each cross of the four crosses *viz.*, Valencia C (P1) × ICGV-SM 02501 (P2), Valencia C (P1) × SGV-07009 (P2), NuMex-M3 (P1) × ICGV-SM 02501 (P2) and Redbeauty (P1) × ICGV-SM 03590 (P2) were set in a randomized complete block design (RCBD) in three replicates with 2-row-plots of ten plants each. The populations and parental lines were planted in the field at a spacing of 45 cm × 15 cm, and the experiment was kept free of weeds throughout the cropping season. The groundnut line JL 24 which is highly susceptible to late leaf spot was used as a spreader row to maintain the effective inoculum load.

Data Collection

Late leaf spot disease severity scoring was done at 115 days after planting using a modified nine-point scale (Subrahmanyam *et al.*, 1995) for all populations of each of the four crosses that were made. Measurements were taken on the following morphological traits that included; Growth habit, number of branches per plant, peg colour, stems pigmentation, stem hairiness, pod reticulation, pod constriction, pod beak and seed characteristics following the International Board for Plant Genetic

Resources (IBPGR, 1992) groundnut descriptor guidelines (Table 1). The data was collected on healthy plants on only populations of Valencia C (P1) × ICGV-SM 02501 (P2) and NuMex-M3 X ICGV-

SM 02501 crosses. The populations from other crosses were high affected by groundnut rosette and no healthy pods available for data collection hence were excluded for further analysis.

Table 1. Botanical types, Origin, pedigree, and response to LLS of six groundnut lines

Genotype	Botanical types	Pedigree	Origin	Response to LLS
Redbeauty	Valencia	Landrace	Uganda	Susceptible
Valencia C	Valencia	Selection from Colorado Manfredi	USA	Susceptible
NuMex-M ₃	Valencia	Valencia C × ICGV 87157	USA	Susceptible
JL 24	Spanish	-----	India	Highly susceptible
ICVG-SM O3590	Virginia	-----	Malawi	Resistant
ICGV-SM 02501	Spanish	-----	Malawi	Resistant
SGV 07009	Virginia	SGV 91707 × Serenut 1	Uganda	Resistant

Table 2. Description of the morphological traits

Trait	Stage recorded	Score
Growth habit	Recorded at podding stage at 10-15cm inter plant spacing	1.Procumbent-1; 2.Procumbent-1 3. Decumbent-1; 4. Decumbent-2; 5. Decumbent-3; 6. Erect
Number of branches per plant	Numbers	Primary (n+1) Secondary(n+2) Tertiary
Peg colour	Recorded on plants after 50% flowering	0 = Absent 1=Present
Stems pigmentation	Recorded on mature plants	0 = Absent 1=Present
Stem hairiness	Recorded on mature plants	0 = Absent 1=Present
Pod beak	Recorded on dry pods	0=Absent/None, 3=Slight 5=Moderate, 7=Prominent 9=Very Prominent
Pod beak reticulation	Recorded on dry pods	0=Absent/None, 3=Slight 5=Moderate,

Pod constriction		7=Prominent 9=Very Prominent 0=Absent/None, 3=Slight 5=Moderate, 7=Prominent 9=Very Prominent
Seed characteristics		1.One colour 2. Variegated
Late leaf spot disease	At harvest on a Subrahmanyam <i>et al.</i> , 1995 scale of 1-9	1: No disease (0%), 2: 1-5%, 3: 6-10%, 4: 11-20%, 5: 21-30%, 6: 31-40%, 7: 41-60%, 8: 61-80% and 9: 81-100%

Analysis

Data taken on the generations of each cross were subjected to analysis of variance (ANOVA) using GenStat version 13 software to test for the significance of the differences between the generations' means of each cross for the LLS disease scores. The generation means were compared using Fisher's protected least significant difference test at 5% level of probability (Payne *et al.*, 2010). Descriptive statistics were used to generate the mean for descriptors. Hierarchical cluster analysis was performed following the unweighted pair group method of the average arithmetic procedure [UPGMA] based on

Euclidean coefficient using GenStat computer package [13 the edition Version 13.3.

RESULTS

Late Leaf Spot Resistance Trait

ANOVA for the four crosses NuMex-M3 × ICGV-SM 02501, Valencia C × ICGV-SM 02501, Redbeauty × ICGV-SM 03590 and Valencia C × SGV-07009 showed significant differences among generations for LLS scores ($P \leq 0.01$) (Table 3).

Table 3. Results of LLS mean score for the six generations of the four crosses.

Generation	NuMex-M ₃ × ICGV-SM 02501	Valencia C × ICGV-SM 02501	Valencia C × SGV 07009	Redbeauty × ICGV-SM 03590
P1 (S)	6.79±0.25c	7.44±0.38d	7.29±0.61b	7.00±0.41c
P2(R)	3.42±0.18a	3.40±0.16a	8.36±0.31b	3.50±0.50a
F1	3.50±0.50ab	3.83±0.40ab	7.52±0.78b	4.50±0.50a
F2	5.33±0.88b	5.22±0.40c	5.00±0.38a	4.60±0.40a
BC1P1	5.25±0.75b	4.75±0.48bc	8.17±0.65b	5.00±0.58ab
BC1P2	4.75±0.63ab	4.25±0.63abc	5.25±0.37a	4.50±0.50a
MS	27.32	20.14	21.20	4.03
F	19.93**	20.80**	13.66**	4.35**
CV %	22.1	20.6	18.2	18.1

P1(S) =Susceptible parent, P2(R) =resistant parent, F1=first filial generation, F2= Second filial generation, BC1P1 = Backcross to susceptible parents (P1) and BC1P2 =Backcross to resistant parent (P2), MS=Mean sum of square, F = Variation ratio, **=significant at $P < 0.01$, CV=Coefficient of variation.

Table 4. Mean of morphological characteristics for the populations of two crosses

Generations	Growth habit	Branch number	Stem pigmentation	Peg colour	Stem hairiness	Pod beak	Pod reticulation	Seed colour	Seed number/pod	Pod constriction
NuMeX-M3 × ICGV-SM 02501										
BC1P1	4.75	13.00	0.00	0.00	0.00	0.00	3.00	1.00	1.00	0.00
BC1P2	3.88	6.75	0.50	1.00	0.75	4.33	2.00	1.00	1.67	0.00
F1	4.80	10.00	0.33	0.33	0.33	5.00	4.33	1.00	2.00	3.67
F2	3.33	9.83	0.25	0.25	0.25	1.00	1.00	1.00	2.33	0.00
ICGV 02501	4.00	23.00	1.00	1.00	1.00	9.00	9.00	2.00	2.00	8.60
NuMeX-M3	6.00	4.50				0.00	0.00	1.00	3.00	0.00
Valencia CX ICGV 02501										
BC1P1	4.67	13.75	0.50	0.25	0.50	1.80	2.40	1.00	2.60	1.20
BC1P2	4.00	20.00	0.50	0.00	0.50	6.60	5.80	1.60	2.40	7.40
F2	4.00	18.33	0.33	0.00	1.00	4.60	5.80	2.00	2.60	6.20
ICGV 02501	4.00	23.00	1.00	0.00	1.00	9.00	9.00	2.00	2.00	8.60
Valencia C	6.00	4.00	0.00	0.00	0.00	1.20	1.20	1.00	3.00	0.60

In the crossing that involved Valencia C and ICGV-SM 02501 parental lines, the susceptible parental line Valencia C had erect growth habit with an average of four branches/plant and had no peg colour, stem pigmentation, and hairs while ICGV-SM 02501 had a decumbent growth habit with an average of 23 branches/plant and with peg colour, pigmented and hairy stems. The F2 population had 18 branches, followed the decumbent growth habit with hairy stems but lacked peg colour and stem pigmentation. The backcross to the susceptible parent (BC1P1) had 14 branches, hairy and pigmented stems with no peg colour and segregated towards an erect growth habit. The BC1P2 populations had 20 branches, hairy and pigmented stems with no peg colour and segregated

towards the decumbent growth habit (Table 4). The susceptible elite variety had no pod constrictions, pod beaks, and reticulations on their pods but had three red seeds/pod on average. For pod and seed characteristics, the backcross population (BC1P1) to the susceptible elite variety exhibited no beak, reticulation, and constriction on their pods like the elite variety and as well segregated towards the three red seeded traits of the elite variety (Plate 1 and 2). The backcross population (BC1P2) exhibited prominent constriction, moderate beak and reticulation on their pods and had on average two tan seeded pods (Plate 1 and 2). The F2 populations exhibited moderate constriction, beak, and reticulation on their pods and had on average three tan seeded pods.



Plate 1



Plate 2

In the NuMeX-M3 \times ICGV-SM 02501 cross, the donor and recurrent exhibited decumbent and erect growth habit respectively. Most of the F2 and BC1P2 plants showed more decumbent growth habit of the donor plants whereas BC1P1 plants segregated more towards the recurrent parent (Table 4). The recurrent parental and donor lines had an average branch number of 5 and 23 per plant. All the segregating populations exhibited branch numbers less than that of the donor's parent. With the exception of BC1P2 population, all other populations of

this cross had lacked stem pigmented and hairs, and peg colour like the NuMeX-M3 line. The donor parent had pods with very deep constriction, and prominent pod beak and reticulation whereas the NuMeX-M3 parental lines had no constrictions, beaks, and reticulations on their pods. Implying that most of the population segregated towards the recurrent parents. On average the NuMeX-M3 parental lines had three red seeds/pod while the donor line ICGV-SM 02501 had 2 tan seeds/pod. All the segregating populations exhibited only two red seeded pods.

Cluster Analysis

For the cross between Valencia C and ICGV-SM 02501 (Figure 1), the similarity between the parental line Valencia C and other generations including the resistant parental line was approximately 50%. The population BC1P1 was approximately 75% similar to Valencia C line. The populations BC1P2 and F2 populations were approximately 95% similar and

approximately 87% similar to ICGV-SM 02501 the resistant line. For the cross NuMex-M3 X ICGV-SM 02501 (Figure 2), the resemblance between the parents based on the analysed traits was approximately 41%. The populations F1, BC1P1, and F2, were approximately 85% and 80 % similar to the recurrent line NuMex-M3 respectively.

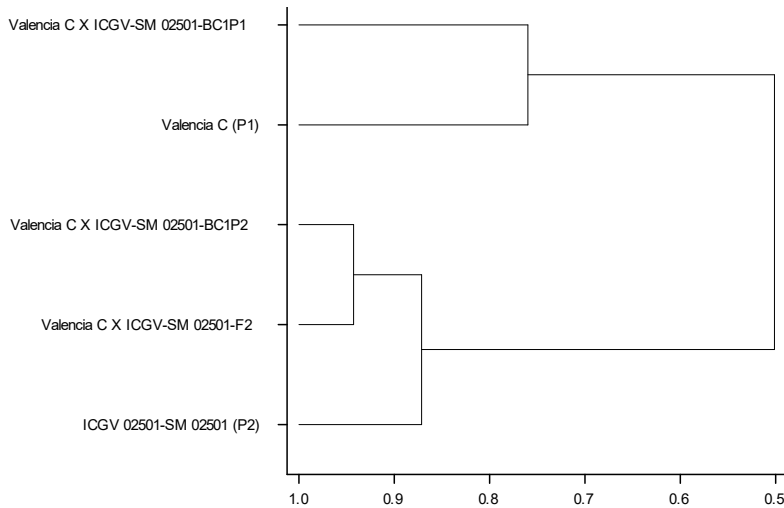


Figure 1. Dendrogram of genetic distances among populations of Valencia C × ICGV-SM 02501 cross

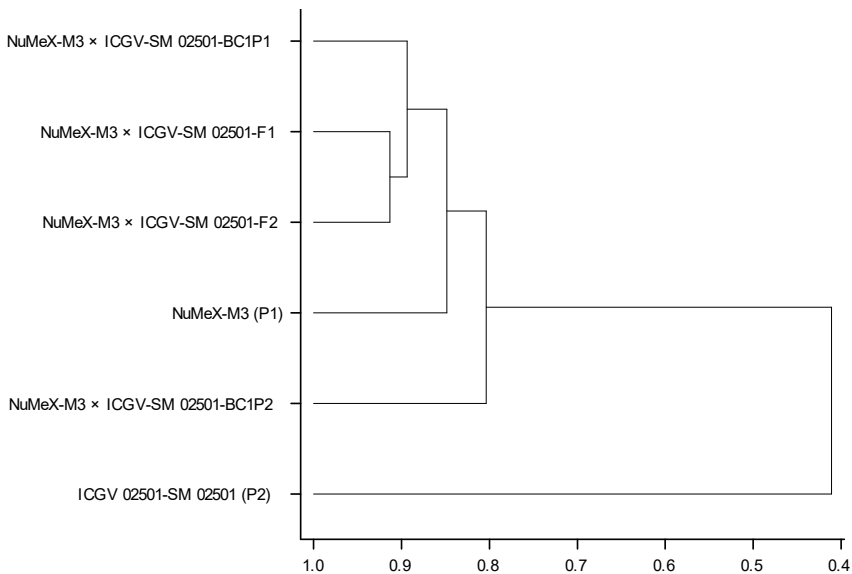


Figure 2. Dendrogram of genetic distances among generations of NuMex-MB × IOGV-SM02501 cross

DISCUSSION

Moderate to high levels of LLS resistance was observed in all populations of the crosses. The resistance that was exhibited among the populations of the crosses depended on the genetic background of the parents that were used in the hybridization. In general, the backcrosses, BC1P1 and BC1P2 showed the mean LLS disease score close to recurrent and donor parents respectively Table 2. The F2 population of the crosses showed moderate highly resistant to LLS. This considerable amount of variability for LLS resistance among the different populations demonstrates additional opportunities for improving groundnut for resistance to LLS through selection. The parental line, SGV-07009 exhibited non-significant difference for late leaf spot disease scores as compared with Valencia C the recurrent parent.

Consequently, its derived populations succumbed to LLS disease except for F2 and BC1P2. Therefore, its populations were excluded for further analysis due to lack of healthy plants for observation. Such progenitor (SGV-07009) should be excluded from the breeding program for LLS resistance because of its weak performance. Similarly, though promising moderate to high LLS resistant segregating populations were recorded from Red beauty x ICGVSM-03590 cross, their podding was highly affected by groundnut rosette disease, and were also excluded for further analysis. These promising populations can be improved by pyramiding LLS and groundnut rosette genes. Though line ICGVSM-03590 combined well for LLS resistance, its combining ability for groundnut rosette was poor with Red beauty. The line exhibited high tolerance to groundnut rosette disease compared to its derived populations. Additionally, it has been reported as a potential donor for groundnut rosette disease Okello *et al.*, (2010). Hence it's important to test such a

good LLS progenitor for its combining ability with other genetic parental background to ensure dual transfer for LLS and groundnut rosette resistance. Nalugo *et al.*, (2016) concluded that heritability of a trait is a property of the genetic background of a population being studied and the environment circumstances to which the individuals are subjected.

The Cluster analysis revealed moderate to high recovery of Valencia traits among promising early segregating LLS resistant populations of NuMex-M3 X ICGV-SM 02501 and Valencia C X ICGV-SM 02501 crosses with major and sub-clusters (Fig 1 and 2). This indicated the various segregation and recombination levels that occurred which contributed to significant variability among the generations for the analysed morphological Valencia traits that could offer possible opportunities for selection in the populations. Pattee *et al.* (2001) reported that segregation within crosses provides additional opportunity for progress from the selection. The results are comparable to those of Kroonenberg *et al.* (2012) who observed morphological variation in section *Arachis*. (Garba *et al.*, 2015) also revealed four distinct groups for hundred groundnuts (*Arachis hypogaea* L.) varieties based on Hierarchical Clustering (AHC) and Fisher Discriminant Analysis (FDA) using twenty-four (24) agromorphological traits.

All the parents that were used to generate crosses had contrasting morphological traits which add more evidence that the parental lines were distinct and belonged to different groundnut botanical varieties. The recurrent parental lines NuMex-M3 and Valencia C belong to *fastigiata* (Valencia type) while ICGV-SM 02501 belongs to *vulgaris* (Spanish type). The results are comparable to those of Krapovickas and Gregory, (1994) who reported four groundnut botanical varieties based on morphological characterization, *fastigiata*

(Valencia type), *vulgaris* (Spanish type), *peruviana*, and *aequatoriana*

In the current study, the genetic distance between the parental lines was approximately 50% and 59 respectively for Valencia C X ICGV-SM 02501 and NuMex-M3 X ICGV-SM 02501 crosses (Figure 1 and 2). This great distance could have contributed to the maximum vigour that was observed in the populations that were derived from these divergent progenitors. The results are in agreement with that of (Oliveira & Valls, 2003) who reported that the most vigorous hybrid in field experiments originated from the parental accessions that verified between the most morphologically divergent progenitors, *A. pintoi* GK 12787 and *A. repens* Nc 1579. The accessions GK 12787 and Nc 1579 that presented the maximum Euclidian distance (5.52) resulted to most vigorous hybrids compared to V 13468 and Nc 1579 which exhibited minimum Euclidian distance (0.20). According to Nalugo *et al* (2014), genetic distance can be an adequate parameter for choosing parental combinations in a crossing program peanut accessions for improving the Valencia background that have a relatively higher number of seeds (4-5 seeds/pod). Therefore, these more distinct lines could be recommend for use as parents in hybridisation for improving Valencia groundnuts for LLS resistance.

In the cross between NuMex-M3 X ICGV-SM 02501, the populations BC1P1 and F2, exhibited higher recovery (approximately 80 %) of the recurrent parental (NuMex-M3) traits. Similarly the backcross population (BC1P1) to the susceptible elite variety of the Valencia C X ICGV-SM 02501 cross exhibited no beak, reticulation and constriction on their pods like the elite variety and as well segregated towards the three red seeded trait of the elite variety and hence recovered more of the recurrent parental traits (approximately 75% similarity to Valencia C) with lower

LLS disease score. Such populations could result in higher gain in Valencia traits and LLS resistance when utilized in the breeding program. The finds confirm to that of (Nalugo *et al.*, 2014) who reported moderate to the high recovery of Valencia traits in crosses that involved Valencia and Spanish botanical types.

The backcross population (BC1P2) of Valencia C X ICGV-SM 02501 cross had reduced prominent constriction, moderate beak and reticulation compared to donor lines that shown very deep prominent beak, reticulation, and constriction on their pods with very low LLS disease score. Suggesting that population slightly segregated to towards the elite variety which grantee opportunities for improvement such a population with high resistance to LLS in advanced stages of selection. More backcrosses can be made to fix the Valencia traits in the advanced hybridization breeding program. According to Poehlman and Sleper (1996), the backcross method is important in recovering the recurrent parent traits in the populations after hybridization.

Conclusion and Recommendation

In the current study, it was revealed that early segregating populations of crosses form genetically diverse populations that offer possible opportunities for selection and enhancing genetic gain in Valencia traits and LLS resistance when advanced in the breeding program. For maximum gain in LLS resistance and recovery of Valencia traits, more selfing and backcrossing can be performed to fix the genes for LLS resistance as well those of Valencia characteristics.

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