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Molecular Characterization and Resistance Profiling of Groundnut Genotypes to Leaf Spot Disease Using SSR Markers

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Abstract

Late Leaf Spot (LLS), caused by *Nothopassalora personata*, is a major fungal disease that significantly reduces peanut (*Arachis hypogaea* L.) production, particularly in Sub-Saharan Africa, including Uganda. This disease lowers the quality of the produce and leads to severe yield losses. Despite control measures, genetic resistance in local peanut cultivars remains limited, and effective management strategies are not well established. Therefore, genetic improvement using LLS resistance is crucial for enhancing peanut productivity. This study aimed to characterize and profile resistance of 25 ICRISAT peanut genotypes, introduced from Malawi and evaluated under Ugandan environmental conditions, using Simple Sequence Repeat (SSR) markers. Genomic DNA was extracted from leaf samples using an optimized Cetyltrimethylammonium Bromide (CTAB) protocol, quantified via spectrophotometry, and amplified by Polymerase Chain Reaction (PCR). Eight SSR markers previously associated with LLS resistance were used to assess their ability to differentiate resistant genotypes. Results revealed that five markers (PM375, PMc588, pPGPseq2B10, pPGPseq2F5, pPGseq17F6) were strongly correlated with LLS resistance, while one marker (pPGseq13A7) was monomorphic. Nine genotypes (36%) carried at least one resistance allele, with five genotypes (20%) classified as resistant, containing four or more resistance alleles, including ICGV-SM 16605, ICGV-SM 16615, ICGV-SM 16602, ICGV-SM 16613, and ICGV-SM 16637. Multivariate analyses, including scatter plots, Unweighted Pair Group Method with Arithmetic Mean (UPGMA) phylogeny, and heat-map clustering, clearly separated genotypes into resistant, tolerant, and susceptible groups. This study highlights the potential of Marker-Assisted Selection (MAS) in identifying and utilizing resistant genotypes for breeding LLS-resistant peanut cultivars in Uganda. Identifying these resistant genotypes offers a valuable resource for improving disease resistance and enhancing sustainable peanut farming production in Uganda and other regions of Sub-Saharan Africa.

Keywords

Late Leaf Spot, Introgression, Marker Assisted Selection, Resistance, Profiling

1. Introduction

Groundnut (*Arachis hypogaea* L.), also known as peanut, is an essential crop worldwide, known for its seeds, oil, and animal feed. It ranked 4th among oilseed crops, contributing significantly to the agricultural economies of both developed and developing countries. Groundnut production is vital for food security and economic stability, and it is a source of employment, especially in developing nations [1]. In Sub-Saharan Africa, groundnuts are particularly important to smallholder farmers, serving as a key source of nutrition and income. These farmers rely on groundnut cultivation to support their livelihoods, making its production a crucial part of rural economies in the region. Uganda, an important East African groundnut producer, faces numerous challenges in maintaining productivity and sustainability within its farming systems. Among these challenges, biotic stresses, especially fungal diseases, pose significant barriers to production. Late Leaf Spot (LLS), caused by the fungus, *Nothopassalora personata*, is one of the most damaging diseases affecting groundnuts. Yield losses from LLS can reach as high as 70% for susceptible varieties, severely impacting both the quantity and quality of the harvest [2]. This leads to considerable economic losses for farmers, worsened by the rising costs of disease control methods. In Uganda, the effects of LLS are particularly harmful, as the pathogen attacks the leaves, causing necrotic lesions that hinder photosynthesis, weaken plant growth, and encourage premature leaf shedding. Infected plants show stunted growth and poor pod and seed development, further lowering yield. Since groundnut is a staple food and cash crop in Uganda, the disease worsens food insecurity and considerably affects farmers' incomes [3]. The disease is most problematic during the wet season, as the warm and humid climate creates favorable conditions for *N. personata* to thrive [2]. Smallholder farmers often turn to fungicide applications to control LLS, which come at a significant financial cost and pose risks to both human health and the environment. These challenges highlight the urgent need for sustainable solutions to control LLS and improve groundnut productivity.

The reliance on conventional chemical control methods shows the need to develop LLS-resistant groundnut varieties. This approach would reduce the dependency on harmful pesticides, lower costs, and promote more sustainable farming practices. Breeding for resistance continues to be an important objective towards achieving a long-lasting control strategy; however, a low spectrum of sources of genetic resistance in currently available local germplasm that is at the disposal of groundnut breeders in the regions is an impediment to breeding progress and success [4]. Molecular breeding methods, developed in the past two decades, promise to provide an effective solution to developing disease-resistant cultivars. Molecular markers, including SSR markers have enabled the identification of genetic loci for groundnut diseases like rust and [4]. They are very polymorphic, allowing accurate identification of putative regions modulating potential resistance candidate genes in diverse panels of germplasm [4].

Quantitative Trait Loci (QTL) associated with resistance to LLS in ground nuts have been mapped by several researchers using SSR markers, which enable application of Marker-Assisted Selection (MAS) procedure in the breeding programmes [2]. Genome-Wide Association Studies (GWAS) and linkage mapping have recently identified major loci for resistance to LLS that can be used in breeding resistant varieties [5]. These technologies have, in the past, facilitated the identification of genetic markers for marker-assisted breeding programs to introduce genes from exotic germplasm-based resistance genes into locally adapted cultivars [6]. Nevertheless, there is still a significant challenge to using these markers in different breeding programmes. This is largely due to lack of fidelity of these markers to genetic background of local germplasm. Besides, there exists a high genetic variability of the groundnut found in Sub-Saharan Africa, in addition to variability presented by ecological environments where groundnut is grown. This results in inconsistent disease expression, which may limit the utilization of markers developed from an exotic genetic background and tested elsewhere [7].

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has been a pioneer in groundnut breeding and has developed a substantial amount of germplasm with differing levels of resistance to LLS. The ICRISAT breeding programme has focused on enhancing the productivity of groundnuts and disease resistance, especially in the arid and semi-arid tropics where groundnut is cultivated as a staple food crop [7]. However, introduced germplasm needs extensive testing to confirm their gene expression for LSS resistance, stability and suitability for production under local conditions. Such germplasm is a potentially useful source of resistance to local breeding programmes. Despite the progress in the LLS resistance breeding by ICRISAT in collaboration with national research systems within the Sub-Saharan region, there is still a lack of evidence on such efforts to deploy resistance genes to improve cultivars locally adapted to Ugandan conditions. The lack of research effort in determining the genetic variability of groundnut landraces in Uganda has further slowed the progress of developing locally adapted, high-yielding, and disease-resistant lines. Currently, studies to deploy SSR markers to assist in resistance breeding in Uganda are scanty. The nature of fungal pathogens' ability to mutate and form new variants calls for the constant search for new germplasm sources with the potential to find new sources of resistance to prevalent diseases [8]. Further, such efforts could be combined with validation of available molecular markers that would enable speedy integration of resistance genes into locally adapted cultivars [9]. Such efforts portend to enhance the productivity and sustainability of groundnut production by developing high-yielding, disease-resistant groundnut cultivars with broad adaptability to the Ugandan agro-ecological system.

However, breeding for LLS resistance has proved challenging due to limited genetic information in African groundnut germplasm and the narrow genetic base of resistance in existing cultivars [1]. Recent advancements in molecular breeding techniques, including the use of Simple Sequence Repeat (SSR) markers, have facilitated the identification of genetic loci linked to disease resistance. These markers have become valuable tools in Marker-Assisted Selection (MAS), allowing for the development of disease-resistant groundnut varieties. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has developed several groundnut genotypes with varying levels of resistance to LLS. Among these, 25 genotypes were pre-selected for their rust resistance and evaluated for their potential resistance to LLS. However, their performance under Ugandan conditions has not been fully assessed. This study aimed to characterize and profile the LLS resistance of these 25 ICRISAT peanut genotypes, introduced from Malawi, and evaluate their resistance under Ugandan environmental conditions using SSR markers. The objective was to improve local breeding programs by incorporating these resistant genotypes into Uganda's groundnut farming systems, enhancing disease resistance and promoting the sustainable production of groundnuts in the region.

2. Materials and Methods

2.1 Plant Materials and Sampling

A total of twenty-five (25) diverse groundnut lines sourced from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) were used in the study (**Table 1**). These were advanced groundnut lines selected for resistance to rust and nutrient density. All 25 genotypes were grown under natural LLS pressure in the 2024 season A (long rains) at NaSARRI and Nakabango, Uganda. Fourteen-day-old, symptom-free leaflets were harvested and desiccated in silica for DNA extraction.

2.2 DNA Extraction and Quantification

The modified Cetyltrimethylammonium Bromide (CTAB) DNA extraction method was used to isolate high-quality genomic DNA [4]. Briefly, 0.4 g of the sample was finely ground in 1500 μL of CTAB buffer to break open cells and protect DNA. The samples were incubated at 65˚C in a water bath shaker to enhance lysis and remove polysaccharides, followed by centrifugation at 13,000 rpm for 10 minutes. The supernatant (900 μL) was transferred to a new tube and mixed with an equal volume of Chloroform Isoamyl Alcohol (CIA) (24:1) for phase separation. The aqueous phase containing DNA was transferred to a clean tube, and DNA was precipitated with 500 μL of ice-cold isopropanol. The resulting DNA pellet was washed with 70% ethanol, air-dried, and resuspended in sterile nuclease-free water [10]. Modifications involved altering the initial incubation time to one and a half hours, washing twice in CIA, and DNA cleanup using serasil beads.

Table 1. List of groundnut genotypes used in the study and pedigree information.

Lab No.	Sample Code	Pedigree data	Source of the Material
1	ICGV-SM16609	(ICGV 02286 X ICGV-SM 05701) F2-P1-P58-B1-B1-B1-B1	ICRISAT
2	ICGV-SM16603	(ICGV 02286 × ICGV-SM 05701) F2-P1-P50-B1-B1-B1-B1	ICRISAT
3	ICGV-SM16605	(ICGV 02286 X ICGV-SM 05701) F2-P1-P54-B1-B1-B1-B1	ICRISAT
4	ICGV-SM16587	(ICGV 02286 X ICGV-SM 05701) F2-P1-P4-B1-B1-B1-B1	ICRISAT
5	ICGV-SM16597	(ICGV 02286 X ICGV-SM 05701) F2-P1-P31-B1-B1-B1-B1	ICRISAT
6	ICGV-SM16617	(ICGV 02286 × ICGV-SM 01514) F2-P1-P2-B1-B1-B1-B1	ICRISAT
7	ICGV-SM16615	(ICGV 02286 × ICGV-SM 05701) F2-P1-P67-B1-B1-B1-B1	ICRISAT
8	ICGV-SM16589	(ICGV 02286 X ICGV-SM 05701) F2-P1-P14-B1-B1-B1-B1	ICRISAT
9	ICGV-SM16616	(ICGV 02286 X ICGV-SM 05701) F2-P1-P68-B1-B1-B1-B1	ICRISAT
10	JL24	Accession	ICRISAT
11	ICGV-SM16613	(ICGV 02286 X ICGV-SM 05701) F2-P1-P64-B1-B1-B1-B1	ICRISAT
12	ICGV-SM16598	(ICGV 02286 X ICGV-SM 05701) F2-P1-P39-B1-B1-B1-B1	ICRISAT
13	ICGV-SM16614	(ICGV 02286 X ICGV-SM 05701) F2-P1-P65-B1-B1-B1-B1	ICRISAT
14	ICGV-SM01514	(ICGV 93437 X ICGV-SM 93561) F2-P15-P3-B2-B1-B1-B1	ICRISAT
15	ICGV-SM16596	(ICGV 02286 X ICGV-SM 05701) F2-P1-P30-B1-B1-B1-B1	ICRISAT
16	ICGV-SM16612	(ICGV 02286 X ICGV-SM 05701) F2-P1-P62-B1-B1-B1-B1	ICRISAT
17	ICGV-SM16602	(ICGV 02286 X ICGV-SM 05701) F2-P1-P49-B1-B1-B1-B1	ICRISAT
18	ICGV-SM16591	(ICGV 02286 X ICGV-SM 05701) F2-P1-P39-B1-B1-B1-B1	ICRISAT
19	ICGV-SM16637	(Pendo X ICGV-SM 95714) BC1F2-P3-P2-B1-B1-B1-B1	ICRISAT
20	ICGV-SM16592	(ICGV 02286 X ICGV-SM 01514) F2-P1-P6-B1-B1-B1-B1	ICRISAT
21	ICGV-SM16604	(ICGV 02286 X ICGV-SM 05701) F2-P1-P53-B1-B1-B1-B1	ICRISAT
22	ICGV-SM16618	(ICGV 02286 × ICGV-SM 01514) F2-P1-P5-B1-B1-B1-B1	ICRISAT
23	ICGV-SM16608	(ICGV 02286 X ICGV-SM 05701) F2-P1-P257-B1-B1-B1-B1	ICRISAT
24	ICGV-SM16601	(ICGV 02286 X ICGV-SM 05701) F2-P1-P44-B1-B1-B1-B1	ICRISAT
25	ICGV-SM16619	(ICGV 02286 X ICGV-SM 01514) F2-P1-P6-B1-B1-B1-B1	ICRISAT

ICRISAT = International Crops Research Institute for the Semi-Arid Tropics.

2.3 Genomic DNA Quality and Quantity Assessment

The purity and concentration of the extracted DNA were assessed using Nanodrop spectrophotometry and gel electrophoresis. Nanodrop spectrophotometry involved evaluating purity at the A260/A280 wavelength ratio, with a value of 1.8 indicating pure DNA. Gel electrophoresis involved resolving the samples in a 1% prestained agarose gel run at 80 V for 15 minutes. DNA presence and integrity were assessed based on the presence, brightness, and sharpness of the resultant bands [11].

2.4 PCR Amplification and Marker Analysis

A total of eight sets of SSR markers (Table 2) were used to amplify DNA from the 25 genotypes using conventional PCR. The process involved the preparation of a reaction mixture containing the template DNA, specific forward and reverse primers, dNTPs, Taq DNA polymerase, reaction buffer with $MgCl_2$, and nuclease-free water [12]. The reaction was performed in a thermal cycler programmed with the following conditions: initial denaturation at 95˚C for 5 minutes, followed by 35 cycles of denaturation at 95˚C for 30 seconds, primer annealing depending on the specific primer, and extension at 72˚C for 30 seconds. A final extension step at 72˚C for 10 minutes was included, and the amplified products were held at 4˚C before being subjected to agarose gel electrophoresis for visualization [12].

Table 2. SSR primers used to screen groundnut genotype samples for LLS resistance.

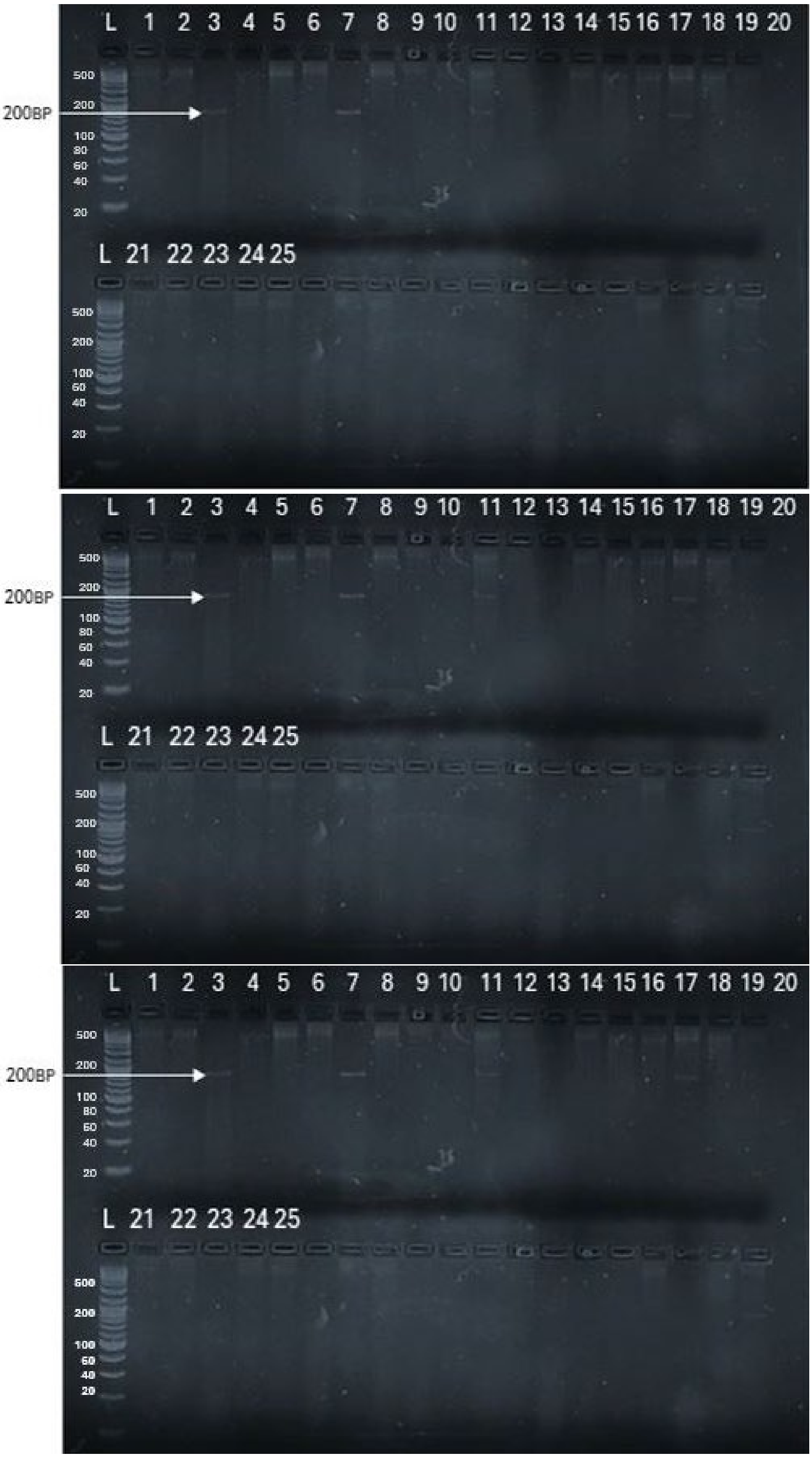
SSR primers	Sequences (5'-3')	Annealing Temperature (°C)
PM383-F	GGCGTGCCAATAGAGGTTTA	52.0
PM384-R	TGAAAACCAACAAGTTTAGTCTCTCT	
pPGpseq5D5-F	AAAAGAAAGACCTTCCCCGA	52.0
pPGpseq5D5-R	ACAGGTAATCTGCCGTGATT	
PM375-F	CGGCAACAGTTTTGATGGTT	55.0
PM375-R	GAAAAATATGCCGCCGTTG	
PMc588-F	CCATTTTGGACCCCCCAAAT	60.0
PMc588-R	TGAGCAATAGTGACCTTGCATT	
pPGPseq2B10-F	AATGCATGAGCTTCCATCAA	50.4
pPGPseq2B10-R	AACCCCATCTTAAAATCTTACCAA	
pPGPseq2F5-F	TGACCAAAGTGATGAAGGGA	50.4
pPGPseq2F5-R	AAGTTGTTTGTACATCTGTCATCG	
pPGseq13A7-F	AATCCGACGCAATGATAAAAA	50.4
pPGseq13A7-R	TCCCCTTATTGTTCCAGCAG	
pPGseq17F6-F	CGTCGGATTTATCTGCCAGT	52.0
pPGseq17F6-R	AGTAGGGGCAAGGGTTGATG	

Source: [13].

3. Results

3.1 Resistant Genotypes and Resistance Patterns

The number of resistance genes identified per genotype ranged from zero to five, and these findings were consistent with the phenotypic results from the field evaluations. Out of the 25 genotypes evaluated, 36% (9 genotypes) exhibited the presence of at least one gene associated with LLS resistance, while 64% (16 genotypes) lacked any resistance genes. However, upon further examination, major genes linked to LLS resistance were detected in two genotypes (**Table 2**), which aligned with field observations. Notably, genotypes ICGV-SM 16605 and ICGV-SM 16615 exhibited the highest number and most diverse types of resistance markers (**Table 2**) and demonstrated the best phenotypic performance in the field, scoring between 2 and 3 on the phenotypic evaluation scale of 1 - 9 [14]. Markers 385F/384R and PGD5D5 were observed in very few genotypes, suggesting that the linked resistance genes may be rare in the materials assessed. Markers PM375, PMc588, pPGPseq2B10, pPGPseq2F5, and pPGPseq17F6 were the most detected markers in the assessed genotypes (ICGV-SM 16605, ICGV-SM 16615, ICGV-SM 16602, and ICGV-SM 16613). **Figure 1** presents gel image amplification of resistant genotypes using markers A, B, C, D, E, and F.



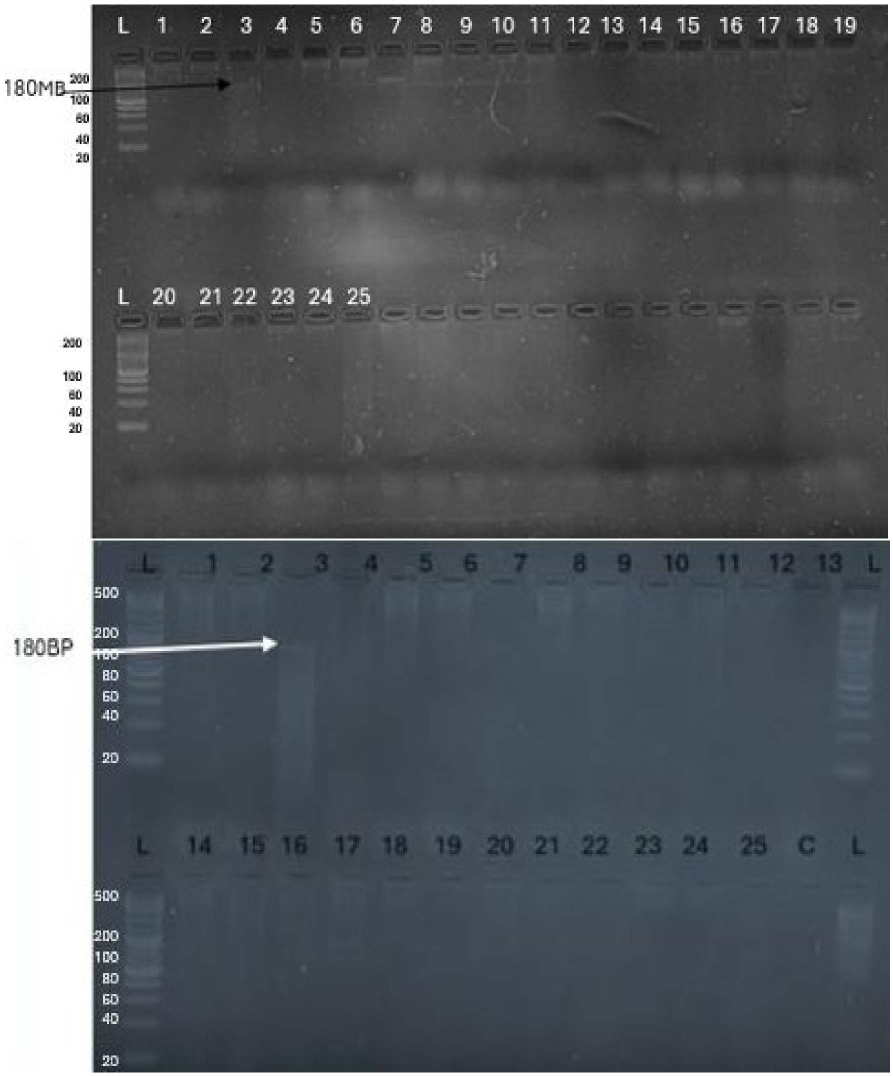


Figure 1. Gel eletrophoresis image showing amplification of resistant genotypes using marker A = PMc375; B = pmC588; C = pPGPseq2B10; D = PM385; E = pPGP17F6 and F = PGD5D5, respectively. L = 500 bp Ladder, 1 - 25 = test samples (Table 2).

Four major genes identified by the following markers: PMc385/384, PGD5D5, pPM375, and PMc588 were detected in 9 genotypes (ICGV-SM16614, ICGV-SM16619, ICGV-SM16692, ICGV-SM16604, ICGV-SM16605, ICGV-SM16615, ICGV-SM16602, ICGV-SM16613, and ICGV-SM16637). The number of major genes identified per genotype varied between 1 and 2. Two genes identified by markers PM375 and PMc588 were the most frequently identified, being present in 4 genotypes each (Table 3).

A total of four markers, *pPGPseq2B10, pPGPseq2F05, pPGPseq13A7,* and *PGP17F6*, flagging minor LLS resistance genes, were assessed and recognized. The LLS resistance gene linked to marker *pPGPseq13A7* was not found in any of the genotypes assessed. Two genotypes, *ICGV-SM* 16605 and *ICGV-SM* 16615, were found to contain more than one minor gene (Table 3). Two markers (*PGD5D5* and *pPGPseq2F05*) were used to identify linked common regions associated with

Table 3: Resistance profiles of the peanut genotypes with resistant markers associated with LLs. Major gene/QTL; ^{s}Minor genes; ^{P}Common QTLs.

Genotype	PM385F/384R*	PGD5D5*^{P}	PM375*	PMc588*	pPGPseq 2B10^{s}	pPGPseq2F05^{sP}	pPGPseq13A7^{s}	pPGP17F6*	Total
ICGV-SM 16605	-	-	+	+	+	+	-	+	**5**
ICGV-SM 16615	-	-	+	+	+	+	-	+	**5**
ICGV-SM 16602	-	-	+	+	+	-	-	+	**4**
ICGV-SM 16613	-	-	+	-	_	-	-	+	**2**
ICGV-SM 16637	-	-	-	+	+	-	-	-	**2**
ICGV-SM 16614	+	-	-	-	-	-	-	-	**1**
ICGV-SM 16692	-	+	-	-	-	-	-	-	**1**
ICGV-SM 16604	-	+	-	-	-	-	-	-	**1**
ICGV-SM 16619	+	-	-	-	-	-	-	-	**1**

Note: The resistant genotypes in this study scored between 2 and 3 in the phenotypic evaluation in the field, showing some level of resistance.

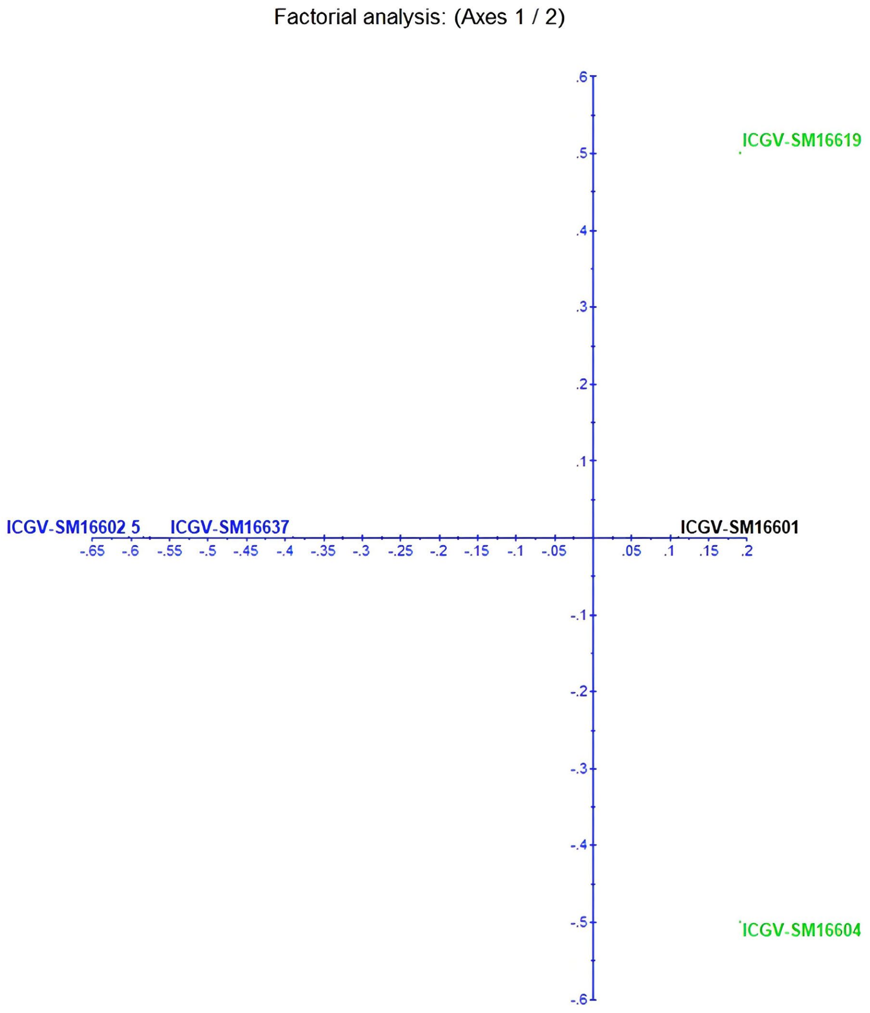


Figure 2. Scatter-plot showing the segregation of the 25 peanut genotypes based on their resistance profiles to LLS.

rust and LLS resistance and were detected in two genotypes each (*ICGV-SM* 16692; *ICGV-SM* 16604 and *ICGV-SM* 16605; *ICGV-SM* 16615 respectively).

3.2 Multivariate Grouping

The scatter plot segregated the genotypes into two clusters based on their resistance to LLS. Cluster I contained genotypes with at least one marker associated with LLS resistance. Cluster II contained all the other genotypes that did not have any marker associated with LLS resistance and were considered susceptible (Figure 2).

3.3 Phylogenetic Analysis

The dendrogram clustered the genotypes into two main clusters. Cluster I contained five genotypes (*ICGV-SM* 16605*, ICGV-SM* 16615*, ICGV-SM* 16602*, ICGV-SM* 16613*, and ICGV-SM* 16637) which had between two and five markers. This cluster contained genotypes that were identified as resistant to LLS. Cluster II contained all the other genotypes that exhibited the presence of one marker or genotypes that did not have any resistance markers to LLS (Figure 3).

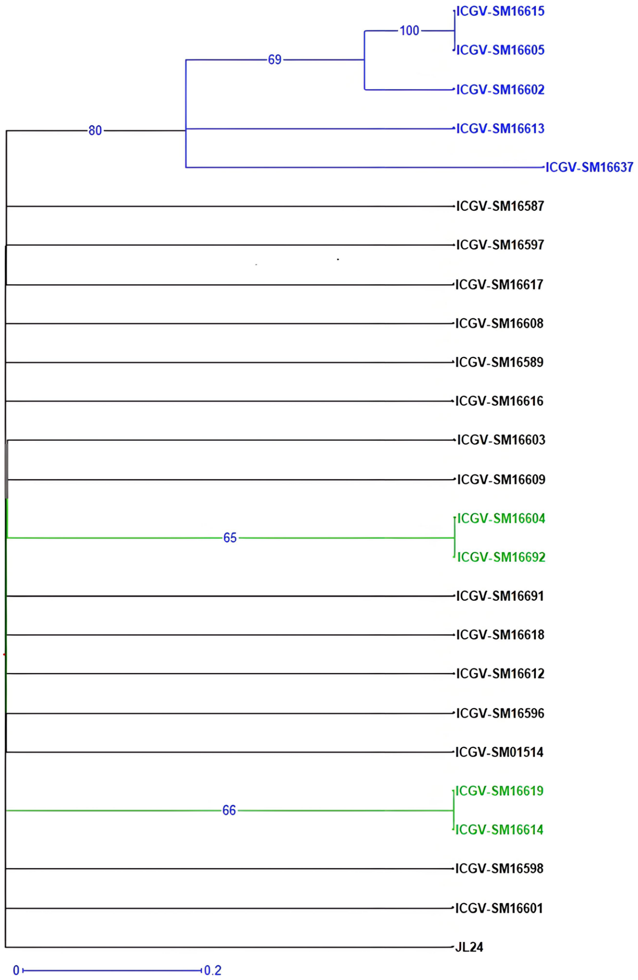


Figure 3. Dendrogram showing clustering of the 25 peanut genotypes based on their resistance to LLS.

Color coding in the figure identify advanced lines based on marker genotypes with Blue = 2 or more resistance makers, Green = one resistance marker and Black = no resistance marker.

3.4 Cluster Analysis of Resistance Profiles

Cluster analysis helped visualize the distribution of resistant genotypes. The results showed that fewer genotypes had multiple resistance-associated markers. This suggests that these genotypes may be relatively resistant to Late Leaf Spot (LLS) disease (**Figure 4**). Notably, genotypes ICGV-SM 16605 and ICGV-SM 16615 had similar resistance profiles, indicating they are likely more resistant to LLS. In contrast, many genotypes lacked most of the tested resistance-associated SSR markers, suggesting they are more susceptible to LLS.

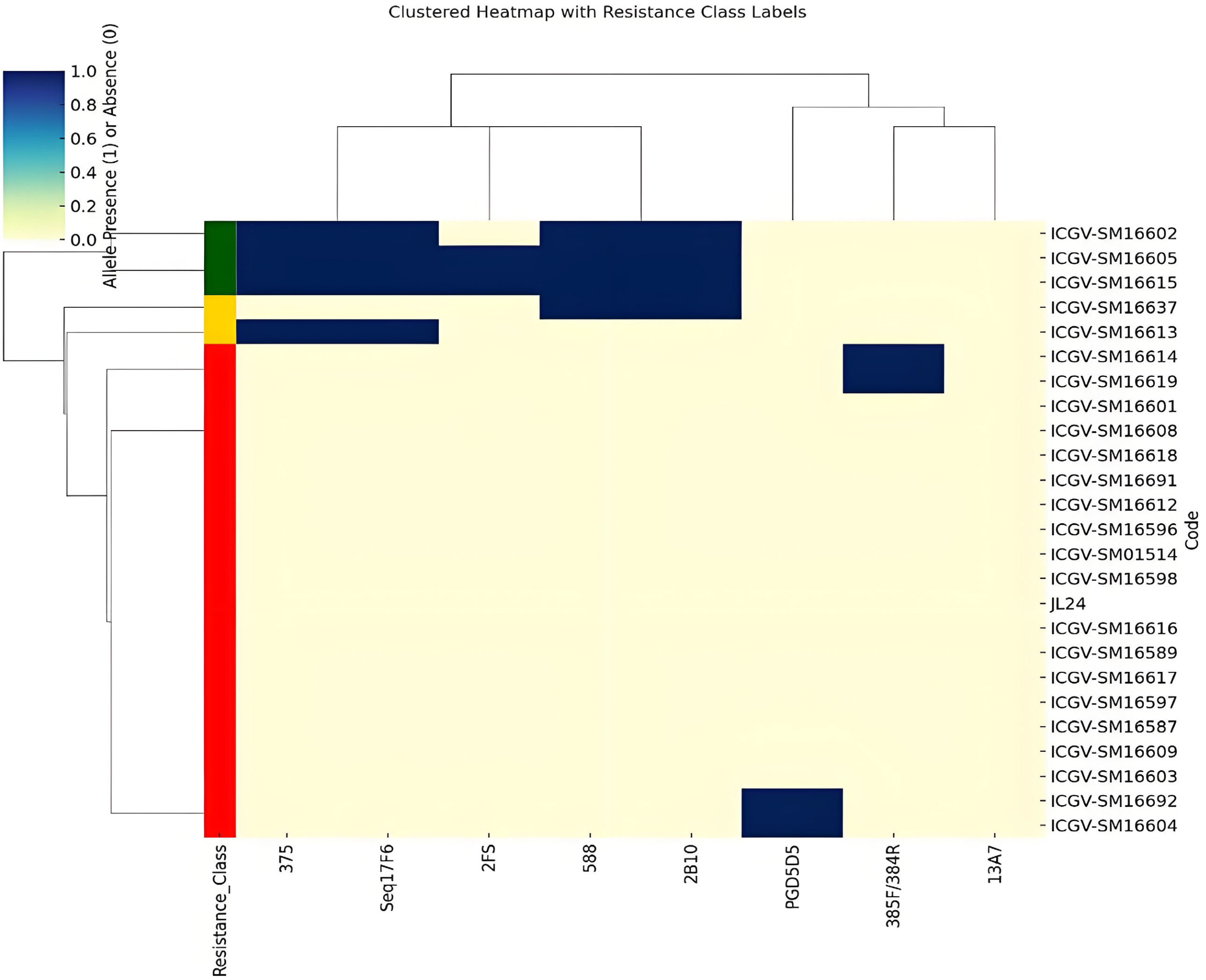


Figure 4. A heat-map visualizing the segregation patterns of the 25 peanut genotypes based on their resistance profiles to LLS.

4. Discussion

Generic resistance to Late Leaf Spot (LLS) in peanuts is a polygenic trait, controlled by both major and minor genes, as demonstrated in this study. Major genes typically confer qualitative resistance, offering a high level of protection against LLS, while minor genes contribute to quantitative resistance, providing more durable and stable protection over time [15]. This polygenic nature of LLS resistance is further supported by the identification of several key markers associated with resistance. The study results revealed that out of the 25 genotypes tested, 36% exhibited the presence of at least one resistance allele, and five genotypes, including ICGV-SM 16605 and ICGV-SM 16615, carried four or more resistance alleles. These genotypes displayed strong resistance to LLS, aligning with the findings of previous studies that indicated the crucial role of both major and minor genes in conferring resistance to the disease [16] [17].

The identification of specific markers, such as PM375, PMc588, pPGPseq2B10, pPGPseq2F5, and pPGseq17F6, strongly correlating with LLS resistance, further strengthens the case for using molecular markers like SSR (Simple Sequence Repeat) markers in Marker-Assisted Selection (MAS) for breeding programs. These markers enable breeders to precisely target genetic loci responsible for disease resistance, thus accelerating the development of resistant cultivars [1]. Notably, marker pPGseq13A7 was monomorphic across all genotypes, indicating that it is less informative for LLS resistance in the studied population and suggesting the need for further research to validate and identify more robust markers for resistance traits. This could be due to a lack of polymorphism within the specific germplasm set tested, indicating that the genetic variation at the locus linked to this marker may be limited in the population evaluated.

Additionally, major genes and minor genes also play an essential role in the expression of resistance. The study identified four minor genes associated with LLS resistance, although not all markers linked to these minor genes were detectable in the genotypes evaluated. Specifically, pPGPseq13A7, linked to minor genes, was absent in all the genotypes tested, while other minor genes like pPGPseq2F5 were found in resistant genotypes, such as ICGV-SM 16605 and ICGV-SM 16615. Minor gene resistance is often more durable than major gene resistance, as it involves multiple genetic loci contributing small effects, which are less likely to be overcome by pathogen evolution [18]. This study supports the importance of integrating both major and minor genes in peanut breeding programs to achieve long-term resistance to LLS.

Multivariate analyses, including scatter plots and phylogenetic trees, clearly segregated the 25 genotypes into resistant, tolerant, and susceptible groups. Genotypes ICGV-SM 16605 and ICGV-SM 16615, which carried the highest number of resistance alleles, clustered in the resistant group, confirming their suitability for use in future breeding programs aimed at enhancing LLS resistance. These results highlight the potential of utilizing molecular tools, such as SSR markers, to identify and select resistant genotypes with both major and minor gene contributions, thereby enhancing breeding efficiency [19]. The heatmap and UPGMA clustering further provided insights into the genetic diversity of the genotypes, revealing that some genotypes with fewer markers for resistance still displayed moderate levels of disease tolerance, thus showing promise for use in breeding programs targeting durable resistance.

In line with findings from previous studies, the results of this research underscore the significance of dual resistance to both LLS and rust in groundnut breeding programs. Markers such as PGD5D5 and pPGPseq2F5, associated with both LLS and rust resistance, were found in genotypes like ICGV-SM 16605 and ICGV-SM 16615, further supporting the utility of dual-resistance breeding. By targeting common QTLs for both diseases, breeders can simultaneously select for resistance to multiple pathogens, reducing the time and cost associated with developing new cultivars [20]. This approach could significantly enhance the efficiency and sustainability of peanut production, particularly in regions like Uganda where both diseases are prevalent.

The complexity of LLS resistance, influenced by a combination of genetic and environmental factors, highlights the need for more comprehensive breeding programs. As this study demonstrates, integrating marker-assisted selection with traditional breeding methods offers a powerful tool for developing groundnut varieties with improved resistance to LLS. However, the effectiveness of these genetic markers in different environmental conditions, such as those found in Uganda, must be further validated. The variability in disease expression and genetic backgrounds among regions emphasizes the need for localized breeding strategies that incorporate both environmental and genetic factors to ensure the successful deployment of resistant cultivars [21].

The identification of resistant genotypes, along with the associated SSR markers, paves the way for more efficient breeding programs aimed at developing high-yielding, disease-resistant groundnut varieties tailored to Uganda's agro-ecological conditions. The findings also reinforce the importance of using a combination of major and minor genes to achieve durable, broad-spectrum resistance, which is crucial for ensuring sustainable peanut production in Sub-Saharan Africa.

5. Conclusions and Recommendations

This study successfully screened 25 ICRISAT groundnut genotypes and characterized their resistance to Late Leaf Spot (LLS) disease under Ugandan conditions using Simple Sequence Repeat (SSR) markers. Five SSR markers were strongly associated with LLS resistance, with nine genotypes (36%) exhibiting at least one resistance allele. Among these, five genotypes, including ICGV-SM 16605 and ICGV-SM 16615, carried four or more resistance alleles, making them promising candidates for breeding programs aimed at improving disease resistance. Multivariate analyses, such as scatter plots and phylogenetic clustering, further differentiated resistant genotypes, reinforcing the potential of Marker-Assisted Selection (MAS) for improving LLS resistance in groundnuts.

Given the polygenic nature of LLS resistance, with both major and minor genes contributing, the study also revealed the possibility of selecting for dual resistance to both LLS and rust, which could significantly enhance breeding efficiency. The identification of such markers offers an opportunity to develop varieties that are resilient to multiple diseases, thereby reducing reliance on chemical control and fostering sustainable agricultural practices.

For future research and breeding, it is recommended that genotypes with confirmed resistance, such as ICGV-SM 16605 and ICGV-SM 16615, undergo multilocation trials to further evaluate their stability and performance in various agroecological zones across Uganda. The study also emphasizes the use of Marker-Assisted Selection (MAS) to accelerate the development of LLS-resistant varieties. Additionally, incorporating dual-resistance strategies, particularly for LLS and rust, should be prioritized in breeding programs to ensure more comprehensive disease resistance. Breeding programs should also explore the integration of resistance genes from wild *Arachis* species, such as *Arachis cardenasii* and *Arachis stenosperma*, to expand the genetic base for LLS resistance. Furthermore, refining phenotyping platforms is crucial to accurately assess both major and minor gene effects under varying environmental conditions. Continued research into the genetic mechanisms behind LLS resistance in local cultivars is needed to optimize breeding strategies. This study, therefore, provides a foundation for future breeding programs focused on developing disease-resistant, high-yielding peanut varieties tailored to Uganda's specific agricultural conditions. The findings contribute to improving groundnut productivity and sustainability in Uganda and other regions of Sub-Saharan Africa.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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