

Evaluation of BC₁F₃ Backcrossed Groundnut (*Arachis hypogea* L) Lines for Resistance to Early and Late Cercospora Leaf Spot in Mali

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Abstract

In Mali, Groundnuts is an important crop for human and animal feeding. However, this crop is suffering from yield losses due to cercospora leaf spot. A study was conducted in Mali to identify lines tolerant to this fungal disease. The present study aims to identify cercospora-tolerant lines. Samples of infested plants, collected at the Kayes research station, allowed for the isolation of the pathogen and the preparation of an inoculum at the IER laboratory in Sotuba. An artificial infestation was then carried out at Kayes research station, based in Samé on Groundnut lines. Analysis of the results revealed a very high incidence but low severity in several lines. However, lines MLGV 2101141, MLGV 2102035, and MLGV 2102046 were resistant under artificial inoculation, with average pod and foliage yields (0.89 t/ha and 1.92 t/ha, respectively) exceeding the average. Four lines moderately susceptible to Cercospora leaf spot (MLGV 2101086, MLGV 2102117, MLGV 2101065, and MLGV 2102126) had higher pod yields. These differences in Cercospora leaf spot tolerance between lines for traits of interest offer an opportunity to select promising lines for the development of new varieties.

Keywords

Groundnut, Cercospora Leaf Spot, Inoculation, Resistance, Yield, Mali

1. Introduction

Early and late cercospora leaf spot, caused respectively by *Cercospora arachidicola* Hori and *Cercosporidium personatum* (Berk and M. A. Curtis) Deighton, are a

major concern for groundnuts, particularly in Mali and generally throughout the Sahel region. The ideal temperature for the proliferation of the fungus responsible for these diseases is between 2°C and 25°C (*Cercospora arachidicola*) and 4°C and 25°C (*Cercosporidium personatum*). The symptoms of cercospora leaf spot are strongly influenced by environmental factors and the genotype of the variety. Small chlorotic spots appear on the first leaves, ten days after inoculum application or at the same time as sowing. Ovoid, raised lesions appear on the green parts (leaflets, petioles, stems) of the plant [1]. Early onset of cercospora leaf spot is characterized by circular spots with irregular borders and diameters ranging from 8 to 10 millimeters. The lesions, dark brown on the upper part of the leaves and paler on the lower part, are regularly arranged in a series of more or less compact wrinkles [2]. Chlorophyll function can be significantly affected due to the extent of rot and leaf drop [3] observed that severe leaf spot damage reduces leaf area by 80%, carbon dioxide (CO₂) uptake by 85%, and the canopy carbon (C) exchange rate by 93%. Photosynthesis in diseased vegetation will be reduced not only by defoliation but also by inefficient carbon dioxide fixation by affected leaves [4].

Studies report that the LLS fungus produces haustoria that penetrate individual plant cells and that leaves infected by the fungus show a marked increase in respiration.

The damage can cause premature plant death [5]. It has a significant impact on pod and haulm yield [6]. When both coexist, losses of 1% to 50% can be estimated [7]. In the Americas, the majority of Groundnut productivity losses can be attributed to damage caused by cercospora leaf spot. In general, in Central Africa, and more specifically in Cameroon, losses of approximately 52% have been observed under experimental conditions. In Nigeria, Côte d'Ivoire, and West Africa, losses range from 30% to 40% and 10% to 30%, respectively [7] [8]. Together with rust, these two types of cercospora leaf spot can cause considerable damage, potentially accounting for up to 70% of pods yield losses [9].

In Mali, a gradual decrease in production of several quintals has been observed in response to various biotic and abiotic stresses, including early black Sigatoka disease. Currently, developing new resistant or tolerant Groundnut varieties is essential to increasing yields in production areas. Given the value of this legume and its economic impact on the income of smallholder farmers, it is necessary to develop new technical approaches to rapidly produce new, highly productive varieties. While various defense methods are employed to eliminate these diseases, the optimal approach remains the creation of new resistant and tolerant cultivars [10]. At the University of Florida in the United States, a highly productive variety, Southern Runner (UF 80202), which is extremely resistant to late black Sigatoka disease, has been developed. ICRISAT has conducted extensive research to create varieties that are resistant to late cercospora leaf spot [2] [11]. Researchers in Mali and Senegal have conducted crosses between resistant lines from Texas and some effective local varieties to create MAGIC (Multi-parent advanced generation inter-cross) populations resistant to black Sigatoka disease. The development of new

varieties will reduce the effects of black Sigatoka on Groundnuts and provide technical information on the disease's progression and the adaptive capacities of new varieties. This study aims to analyze the behavior of 314 genotypes generated by backcrossing at the Kayes research station, through artificial inoculation with an isolate of *Cercospora arachidicola* Hori.

2. Material and Methods

Material

Site:

The trial was set up in the nursery of the Samé-Kayes agronomic research station (longitude 11°34' west; latitude 14°29' north; altitude 281 m). With a tropical ferruginous soil and a weakly rich sandy loam structure, leached with concretions.

Plant Material

The study consisted of 324 entries: 314 lines generated by backcrossing from a donor variety (T4-2006) highly resistant to early cercospora leaf spot and two recipient varieties (Fleur 11 and ICGV86124) susceptible to both forms of cercospora leaf spot. A regional resistant control (ICGV 01276), a local resistant control (ICGV 00064), two susceptible controls (ICGV 86015, ICGV 13825), and four varieties selected from those known and widespread in Mali and the West African sub-region were also included. These were: ICGV 99029; JL 24; 55437; and ICGV 7878. The abbreviation ICGV used for the varieties designates the ICRISAT Groundnut variety, while MLGV designates the Mali Groundnut variety. The first two digits following ICGV and MLGV indicate the year of hybridization, while the next two indicate the cross population, and the last two or three digits are the plot or plant numbers. Diseased plant debris contains the fungal pathogen.

Small Equipment

Chemicals (Tween 20, distilled water, alcohol, muslin), a hemocytometer, and laboratory forceps were used to prepare the isolate. The station is equipped with a photovoltaic irrigation system for off-season use and supplemental watering during dry spells.

Experimental Design

The experimental design used was an 18 × 18 Augmented Design without replication, consisting of contiguous plots spaced 4 m apart in a row, with 0.50 m between planting rows and 0.10 m between planting holes. This resulted in a plot area of 4 m × 0.5 m. Only one factor was studied: the line or variety.

Methods

Observations:

During the pre-harvest period, observations were made in the seed rows and focused on the following parameters:

Emergence Date (Duration of Emergence):

This is the number of days between sowing and emergence of more than 50% of the plants (IBPGR and ICRISAT, 1992). It is determined after manually counting the emerged plants in the seed rows following regular inspections.

Number of Plants at Emergence

This is estimated by visual (or manual) counting of plants with at least two green leaves in the seed rows.

Flowering Start Date

This is the number of days between sowing and the date on which a plant has at least one flower.

Number of Days at 50% Flowering

This represents the number of days between sowing and the day on which 50% of the plants have at least one flower. It is obtained by visual counting in the seed rows.

Foliar Disease Rating

This involved evaluating plant behavior in relation to certain disease pressures. Regarding early and late cercospora leaf spot, the rating was based on a scale of 1 to 9 and applied to the main stem according to the method of Subrahmanyam *et al.* (1982), from no incidence to more than 80% of leaflets damaged:

Genotypes with scores between 1 and 2 are considered resistant, 3 to 4 are considered moderately resistant, and those with a score of 5 to 6 are considered tolerant. Conversely, those with a score above 6 are considered susceptible.

Harvest Date

This is the number of days between sowing and the harvest date.

During the post-harvest period, observations focused on the following parameters:

Number of Plants Harvested

This is the count of the number of Groundnut plants actually harvested per plot.

Haulm Weight

This is the mass of hazelnut from all the Groundnut plants in a single plot. It is obtained by weighing the plants on a scale after air drying (moisture content reduced to approximately 10%).

Number of Pods Per Plant

This involves randomly selecting 5 Groundnut plants and counting them in each plot.

Pod Weight

The mass of pods from a single plot. It is measured using a scale after air drying (seed moisture content of approximately 10%).

Weight of 100 Seeds

This is obtained by weighing the seeds on a randomly selected scale.

Shelling Percentage (DC%):

$$DC\% = \left(\frac{P200}{200} \right) * 100 \quad (1)$$

Disease Incidence:

Observations were made on cercospora leaf spot to determine the incidence and severity of cercospora leaf spot on the different genotypes tested. The incidence,

expressed as a percentage (1%), represents the proportion of infected plants and is calculated using the following formula:

$$I = \frac{n}{N} * 100 \quad (2)$$

Where n represents the number of infected plants and N the total number of plants observed.

Disease Severity

Severity (S) indicates the severity of the cercospora leaf spot, assessed based on the row of an elementary plot. Its value was calculated using the following formula :

$$S = \frac{\sum x_i * n_i}{9 * N} * 100 \quad (3)$$

Where, x_i is the number of infected plants, n_i is the value of the score obtained according to the scale from 1 to 9, where the coefficient 9 is the ceiling, N is the total number of plants per elementary plot.

Trial Procedure

After flat plowing, the plot was delineated using a tape measure, string, and stakes. Delineation began by marking a right angle using the 3-4-5 method. First, the entire plot was delineated, and then within it, the blocks and elementary plots were marked out. These compartments are all made up of stakes providing an overall view of the trial area. After thorough watering, sowing took place 10 days after plowing to avoid weeds and off-types. According to the randomized experimental design, the bags containing the seeds for each of the 324 entries were placed at the beginning (on the row) of the elementary plot.

The seeds were sown by hand in small hills. Each hill contained one seed and was covered with soil, then lightly firmed with hoes. Maintenance was carried out to prevent competition and promote harmonious crop development. This maintenance work was performed as needed throughout the growing season, using a hoe. After the first weeding 20 days after sowing, the soil was hilled to strengthen the soil around the plants and encourage good penetration of the gynophores into the soil. The second hilling was done after adequate watering (14 days after the first).

Other maintenance work was also carried out, mainly using manual weeding when necessary. Diammonium phosphate (DAP: 18-46-0) was applied one month after sowing, following weeding. Based on analyses, applications were made to the individual plots at a rate of 100 kg/ha. The DAP was broadcast in the individual plots in a single day. Harvesting took place three months and one week after sowing, using hoes to lift the soil and pods.

The number of plants at harvest was recorded simultaneously. The corresponding plots were manually destemmed, and the pods were placed in cretonne bags labeled with the names of the different lines, entry numbers, year, and trial name to facilitate identification. The foliage and pods were exposed to the open sun in their respective plots, and the various weights were recorded ten days later. The pods were also air-dried in the sun in a field area for 15 days to achieve a moisture

content of approximately 10%. Subsequently, the various weights were recorded using a precision balance. The foliage was weighed in the different plots using a balance with a maximum capacity of 100 kg. The pods were weighed in the laboratory using an electronic balance to determine their precise masses.

Throughout the biological parameter trial, observations were conducted according to the experimental protocol. The trial was irrigated using the irrigation system until the full onset of rainfall in early July. Data were collected using tablets formatted with an Excel interface for thorough data cleaning. Descriptive and multivariate statistical analyses were performed using R software (ggplot2, lme4 packages) and XLSTAT 2024 with the R language.

Sampling

Samples were collected from experimental fields heavily affected by cercospora leaf spot 80 days after sowing during the off-season at the Samé research station to obtain a representative sample. The site was selected because it is the permanent research center for all Groundnut varieties being disseminated or tested in Mali. Sampling was carried out along the two diagonals of the field at four points, with the final sample taken at the point of intersection of the two diagonals.

Isolation and Identification of the Fungal Isolate

The purified culture medium was collected using a sterilized metal spatula, and the mycelium was then suspended in distilled water and homogenized. Muslin was used to separate the spores from the mycelium residue, and the resulting suspension was filtered to remove the mycelium residue. The number of conidia was measured using a hemocytometer to determine the optimal concentration. The spore concentration was then adjusted using distilled water to achieve a final concentration of 2×10^4 spores/ml.

Application Method

During field spraying, 0.05% of Tween 20 was added to the inoculum to promote spore adhesion to the leaves. The resulting solution was placed in a constant-pressure sprayer and mixed with one liter of distilled water. Inoculation took place eight weeks, or two months, after sowing, in the evening following thorough watering with a constant-pressure sprayer.

Virulence Test of the Fungal Isolate

All the tested varieties and lines showed resistance to Cercospora leaf spot. The spore solution (inoculate) was applied eight weeks after sowing (60 days after sowing) using a continuous pressure sprayer to five randomly selected plants, each designated as a plot.

Data Analysis

The observation results were entered into Excel 2019 and analyzed using XLSTAT 2024 software with an R interface (Ggplot 2 package). The Newman-Keuls test was applied at a significance level of 5% to compare the mean results of the different treatment parameters.

Results, and Discussions

Results

Rainfall Conditions at the Station:

Rainfall was variable but well distributed from month to month, both in terms of the amount of water collected and the number of rainy days. The first ten days of September saw a record accumulation of 138 mm, followed by the second ten days of August with 108 mm (Figure 1). A total of 601.0 mm of rain was recorded over 36 rainy days from July to October. Overall, physiological conditions were favorable for good plant production and productivity. This year, the wettest month was July, with 217.0 mm over 10 rainy days.

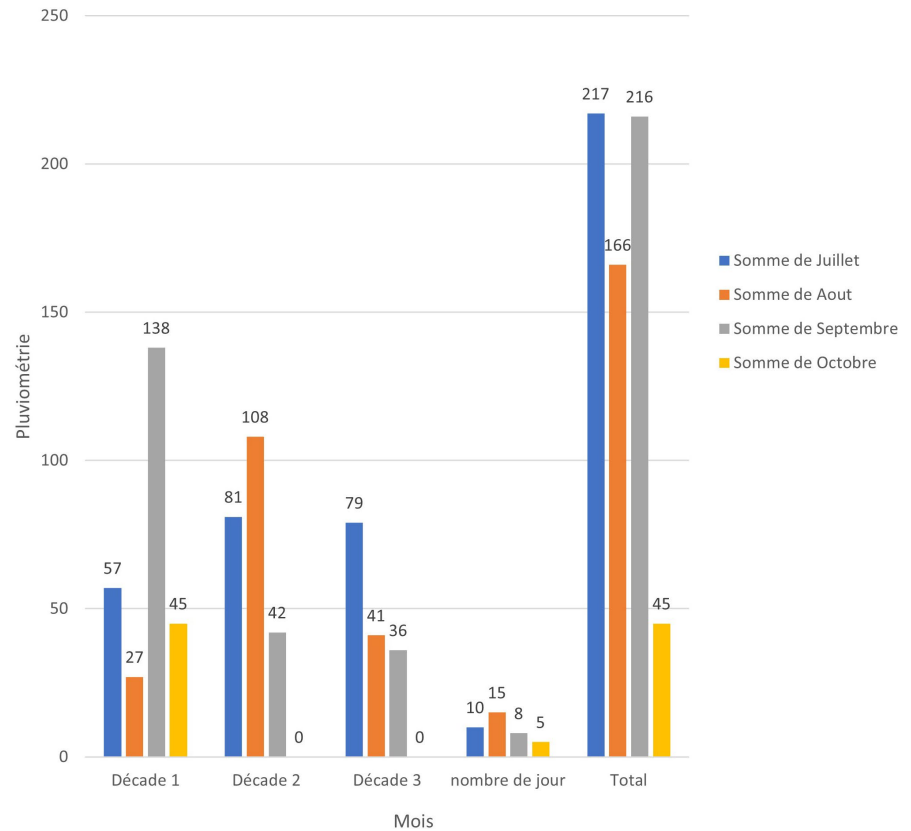


Figure 1. Rainfall amount at the Samé station (year 2024).

The station recorded an average of 685.0 mm. Compared to 2023, with a total of 1086.0 mm over 43 days of rain, it was less abundant.

Summary of Complete Statistical Analyses

Table 1. Descriptive statistics.

Variable	Observations	Obs. avec données manquantes	Obs. sans données manquantes	Minimum	Maximum	Moyenne	Ecart-type	cv
DEM	324	0	324	6.000	9.000	7.099	0.419	0.06
NPL	324	0	324	0.000	66.000	24.099	10.175	0.42
DF	324	0	324	0.000	22.000	17.488	1.666	0.10
DF50%	324	0	324	0.000	25.000	19.960	1.907	0.10

Continued

DM	324	0	324	85.000	100.000	89.735	1.022	0.01
ELS90	324	0	324	1.000	4.000	1.812	0.689	0.38
LLS90	324	0	324	1.000	2.000	1.074	0.262	0.24
NPR	324	0	324	5.000	46.000	20.963	7.623	0.36
NG/P	324	0	324	3.000	42.000	19.861	5.768	0.29
PF(kg)/plot	324	0	324	0.210	2.030	0.770	0.354	0.46
RDTfane (kg/ha)	324	0	324	525.000	5075.000	1924.074	884.723	0.46
P20	324	0	324	8.000	31.000	17.491	3.138	0.18
P100	324	0	324	30.000	95.000	58.025	10.701	0.18
P20GR	324	0	324	6.000	19.000	11.605	2.140	0.18
DC%	324	0	324	38.890	175.000	67.322	12.566	0.19
HI	324	0	324	0.590	68.710	33.815	11.110	0.33
PG(g)/plot	324	0	324	119.000	1190.000	356.491	108.257	0.30
PG(kg)/plot	324	0	324	0.119	1.190	0.356	0.108	0.30
RDTgousse(t/ha)	324	0	324	0.300	2.980	0.892	0.271	0.30
RDTgousse (kg/ha)	324	0	324	297.500	2975.000	891.227	270.642	0.30
AUSPC	324	0	324	4.148	19.852	6.069	1.502	0.25

NB: DEM = emergence date; NPL = number of plants raised; DF = flowering date; DF50 = (50. %) percent flowering; DM = maturation or cycle date; ELS = early leaf spot; LLS = late leaf spot; NPR = number of plants harvested; NG/P = number of pods per plant; RDTfane = biomass of stems, roots and leaves; P100 = weight of 100 seeds; DC% = percentage of hulling or shelling; HI = Harvest index; RDTgousse = pod yield; AUSPC = leaf area destroyed.

Analysis of (**Table 1**) highlights the contrasting results for the tested Groundnut lines. Emergence was rapid and uniform, with emergence observed after an average of seven days. The homogeneity of the lines is evidenced by a low coefficient of variation (CV of 6%). However, the germination rate reached only 53.12% of the theoretical planting density, which constitutes a primary limiting factor for the final yield. The earliness of the lines represents a major advantage. Flowering began 17.5 days after sowing, and by day 23, more than 80% of the plants had flowered. This early and concentrated flowering promoted good fertilization, resulting in a satisfactory number of pods per plant, with an average of 20 pods per plant (within the normal range of 18 to 22) and a maximum of 42 observed. The individual potential of the plants is evident.

However, this individual potential did not fully translate into yield per hectare. The average pod yield was 891.23 kg/ha, a result lower than the species' potential, estimated at between 1200 and 1500 kg/ha. This disappointing performance is explained by the moderate emergence rate and significant heterogeneity in performance between lines, as evidenced by the very high CV of 30%. This wide variability was also observed for the haulm yield, which averaged 1924 kg/ha, but with extremes ranging from 525 to 5075 kg/ha. Finally, a particularly notable and positive result concerns the health status of the crops. The development of both forms

of cercospora leaf spot remained very low, with scores of 1.81 for early cercospora leaf spot, indicating excellent resistance of the lines to this disease.

Table 2 provides numerical evidence for the study's main conclusion: the tested lines exhibit a very positive agronomic profile, characterized by dual performance in terms of resistance to cercospora leaf spot and high yields. It allows for the identification and ranking of the highest-performing lines based on robust statistical data (mean, standard deviation, coefficient of variation, etc.). This is a fundamental decision-making tool that justifies the selection of the best lines for future breeding programs.

Contribution to the analysis of variance

Table 2. Contribution to the analysis of variance (ANOVA table).

Variable	DDL (Modèle)	Moyenne des carrés (Modèle)	DDL (Erreur)	Moyenne des carrés (Erreur)	F	Pr > F	Signification
DEM	1	0.291	322	0.176	1.658	0.199	NS
NPL	1	2670.071	322	95.561	27.941	<0.0001	THS
DF	1	5.682	322	2.768	2.053	0.153	NS
DF50%	1	8.518	322	3.621	2.352	0.126	NS
DM	1	0.806	322	1.045	0.771	0.380	NS
ELS90	1	0.240	322	0.476	0.504	0.478	NS
LLS90	1	0.060	322	0.069	0.872	0.351	NS
NPR	1	2750.394	322	49.743	55.292	<0.0001	THS
NG/P	1	750.166	322	31.045	24.164	<0.0001	THS
PF (kg)/plot	1	27.607	322	0.040	692.092	<0.0001	THS
RDTfane (kg/ha)	1	172545580.786	322	249310.222	692.092	<0.0001	THS
RDTfane (t/ha)	1	75.626	322	40.131	1.884	0.171	NS
P20	1	19.613	322	9.818	1.998	0.159	NS
P100	1	188.927	322	114.276	1.653	0.199	NS
P20GR	1	7.557	322	4.571	1.653	0.199	NS
DC%	1	19.063	322	158.345	0.120	0.729	NS
HI	1	14821.490	322	77.796	190.516	<0.0001	THS
PG (g)/plot	1	89353.384	322	11478.446	7.784	0.006	HS
PG (kg)/plot	1	0.089	322	0.011	7.784	0.006	HS
RDTgousse (t/ha)	1	0.558	322	0.072	7.773	0.006	HS
RDTgousse (kg/ha)	1	558458.649	322	71740.287	7.784	0.006	HS
AUSPC	1	8.813	322	2.236	3.942	0.048	S

Note: S = significant, NS = not significant, HS = highly significant, THS = very highly significant.

Analysis of variance (ANOVA) (**Table 2**) assessed the effect of the different lines tested on a set of agronomic variables. The results revealed very highly sig-

nificant differences (THS, $p < 0.001$) between the lines for several key productivity variables, including the number of emerged plants, the number of plants harvested, the number of pods per plant, the haulm yield in kilograms per hectare, and the Harvest Index and AUSPC. In contrast, no difference (NS) was observed for phenological variables, such as flowering date, ripening date, ELS, and LLS. Overall, it could be said that the experimental conditions strongly impacted specific aspects such as the number of plants, flowering, and aboveground biomass.

Foliar Diseases: Early and Late Leaf Spots

The main foliar diseases observed were early and late leaf spot. Data on these two diseases allowed us to determine their incidence on the different lines tested. Varieties with a score of 1, 2, or 3 were considered resistant, and those with a score of 4 to 5 were considered tolerant. Conversely, those with a score above 5 were considered susceptible.

Most of the lines tested showed resistance to or tolerance of both forms of leaf spot. The analyses showed that not all lines exhibited the same level of manifestation of the two forms of leaf spot. In both cases, the observation was that the number of treatments with a score of 1 was predominant. The most resistant treatments were: MLGV 2101149, MLGV 2102129, MLGV 2101076 (score 1), and MLGV 2102064, with the controls also having a score of 1 (ICGV 00064 and ICGV 7878). In contrast, the varieties [MLGV 2101026, MLGV 2102136, MLGV 2102117, MLGV 2102141, MLGV 2101065] and the control [Fleur 11] showed moderate resistance to *Cercospora* leaf spot, obtaining scores [3 and 4] (**Table 1**). Overall, all the tested lines developed resistance to both forms of *Cercospora* leaf spot.

Pod Yield

The pod yield analysis revealed a significant difference between the tested lines (**Table 2**). The analyses showed a significant difference between treatments, with an average of 891.23 kg/ha and a coefficient of variation (CV) of 30% (**Table 1**). A wide variation was observed in the data distribution, ranging from a minimum of 297.50 kg/ha to a maximum of 2975.00 kg/ha. Line MLGV 2101032, with a yield of 2975.00 kg/ha, was the best, followed by lines MLGV 2101141 (1900.00 kg/ha), MLGV 2102035 (1715.00 kg/ha), and MLGV 2102046 (1660.00 kg/ha). In contrast, among the treatments in the trial, the lines with the lowest pod yield were: MLGV 2102092 (362.50 kg/ha); MLGV 2101032 (385.00 kg/ha); and MLGV 21002099 with a yield of 497.50 kg/ha. The control parent, T4-2006, yielded 1570.00 kg/ha, significantly exceeding most of the lines.

Haulm Yield

The hazelnut yield data in (**Tables 1-3**) and (**Figure 2**) showed a significant difference between treatments at the 5% significance level with a coefficient of variation (CV) of 46% (**Table 1**). Hazelnut yields ranged from 5075.00 to 525.00 kg/ha, with an average of 1924.07 kg/ha. Among the 324 treatments, lines MLGV2102002, MLGV 2102118, and MLGV 2101060 had the highest haulm yields, while the control ICGV 01276 had a good yield of 3750 kg/ha. Lines MLGV

2101092, MLGV 2101135, and MLGV 2101036 had the lowest yields, ranging from 500 to 600 kg/ha. Lines that exhibited good haulm and pod yields could be used as dual-purpose lines. Soil conditions played a role in the lower pod and haulm yields.

Multivariate analysis (PCA and others) Correlations

Table 3. Correlation matrix of the different observed parameters.

de\Vers	DEM	NPL	DF	DF50%	DM	ELS90	LLS90	NPR	NG/P	PF(kg)/plot	RDTfane (kg/ha)	RDTfane (t/ha)	P20	P100	P20GR	DC%	HI	PG(g)/plot	PG(kg)/plot	RDTgousse (t/ha)	RDTgousse (kg/ha)	AUSPC	
DEM	1																						
NPL	0.109	1																					
DF	-0.020	0.029	1																				
DF50%	-0.011	0.034	0.956	1																			
DM	0.235	0.094	-0.038	-0.056	1																		
ELS90	-0.010	-0.017	-0.057	-0.034	-0.040	1																	
LLS90	0.046	-0.043	-0.026	-0.013	0.027	0.488	1																
NPR	0.034	0.356	-0.164	-0.148	-0.015	0.056	0.011	1															
NG/P	0.056	0.034	-0.012	-0.017	-0.005	0.008	-0.003	-0.071	1														
PF(kg)/plot	-0.034	0.327	-0.135	-0.143	-0.051	0.053	0.037	0.445	0.351	1													
RDTfane (kg/ha)	-0.034	0.327	-0.135	-0.143	-0.051	0.053	0.037	0.445	0.351	1.000	1												
RDTfane (t/ha)	-0.018	0.001	-0.002	-0.019	0.007	0.023	-0.010	-0.025	0.002	0.091	0.091	1											
P20	0.041	0.040	-0.124	-0.107	0.045	-0.085	-0.067	0.113	-0.023	0.037	0.037	-0.074	1										
P100	0.023	0.200	-0.075	-0.043	-0.020	-0.048	-0.019	0.083	-0.019	0.090	0.090	0.023	0.546	1									
P20GR	0.023	0.200	-0.075	-0.043	-0.020	-0.048	-0.019	0.083	-0.019	0.090	0.090	0.023	0.546	1.000	1								
DC%	-0.015	0.186	0.027	0.040	-0.086	0.008	0.044	-0.046	-0.002	0.049	0.049	0.117	-0.433	0.477	0.477	1							
HI	0.069	-0.144	-0.037	-0.018	0.104	-0.032	0.006	-0.292	-0.284	-0.763	-0.763	-0.273	0.021	0.006	0.006	-0.016	1						
PG(g)/plot	0.083	0.295	-0.403	-0.387	0.141	-0.004	0.006	0.220	0.040	0.255	0.255	-0.007	0.167	0.236	0.236	0.089	0.268	1					
PG(kg)/plot	0.083	0.295	-0.403	-0.387	0.141	-0.004	0.006	0.220	0.040	0.255	0.255	-0.007	0.167	0.236	0.236	0.089	0.268	1.000	1				
RDTgousse (t/ha)	0.083	0.294	-0.403	-0.387	0.141	-0.004	0.005	0.219	0.041	0.254	0.254	-0.008	0.167	0.235	0.235	0.088	0.269	1.000	1.000	1			
RDTgousse (kg/ha)	0.083	0.295	-0.403	-0.387	0.141	-0.004	0.006	0.220	0.040	0.255	0.255	-0.007	0.167	0.236	0.236	0.089	0.268	1.000	1.000	1.000	1		
AUSPC	0.027	0.079	-0.056	-0.017	-0.036	-0.004	0.006	0.007	0.300	0.204	0.204	-0.032	-0.021	0.060	0.060	0.059	-0.163	0.038	0.038	0.038	0.038	1	

Note. Values in bold red are positively correlated, those in green are negatively correlated (t) and are different from 0 at a significance level ($\alpha = 0.05$).

Compared to the correlation table (**Table 3**), significant correlations between

variables are in bold. Here, correlations were indicated by two colors: green for negative correlations and red for positive correlations.

Thus, strong and significant positive correlations were recorded between emergence date and maturity date (23.5%), ELS and LLS (48.80%), foliage weight and number of emerged plants (32.70%), foliage weight and number of harvested plants (44.50%), and number of emerged plants (NPL) and number of harvested plants (NPR) (35.60%). Significant negative correlations were recorded between the different variables: DF/DF50% and NPR (16.4% and 14.80%); HI (Harvest Index) and foliage weight (76.30%), pod yield and date of first flower appearance (40.30%) / date of 50% flowering (38.70%).

From the analysis of the table, it could be said that when the harvest index (HI) increased, then foliage weight decreased. Conversely, foliage weight increased when the number of plants emerged and harvested increased (**Table 3**).

Compared to the correlation circle, we found that 23.88% of the correlations were explained by axis F1 and 14.7% by axis F2. The variables best represented on axis F1 were flowering date (FD), pod yield, leaf yield, and the 50% flowering date (D50%). Axis F2 better explained the variables Harvest index (HI) and leaf yield. The smaller the separation angle between two variables, the more similar their characteristics. However, do not assume that two variables are negatively or positively correlated, as this is more apparent than real. The Euclidean plane allowed us to find the x and y coordinates of the variables with respect to the x-axis (23.88%) and y-axis (14.7%), in the same proportions as the circle (**Figure 2**).

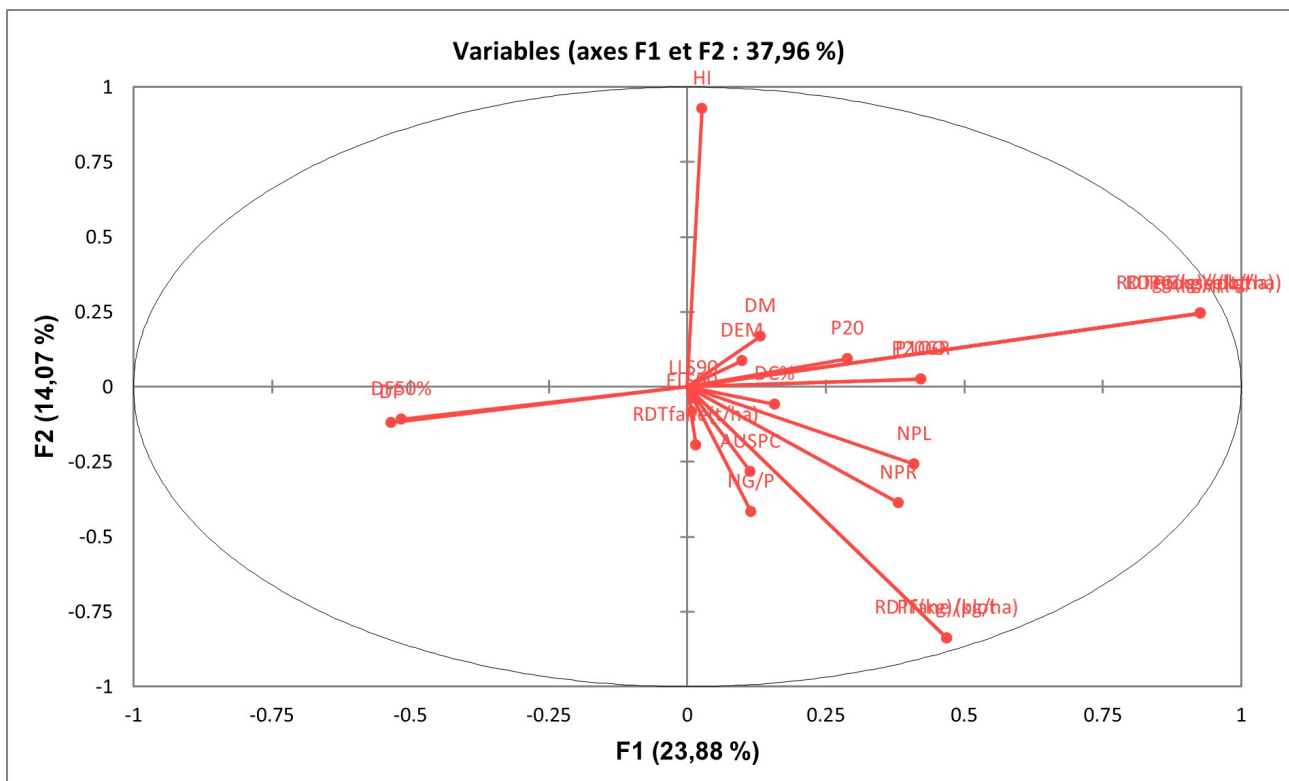


Figure 2. The correlation circle of variables.

Scatter Plot

The graph (**Figure 3**) below corresponds to principal component analysis objectives. It allowed us to represent the treatments on a two-dimensional map and to identify trends. Based on the available pod yield variables, lines MLGV 2102154 /ELS (3) (1570 kg/ha) and MLGV 2102072 (1152.50 kg/ha and ELS (1)) are quite remarkable. Similarly, MLGV 2102002 and MLGV 2101136, both with ELS/LLS scores of 2 and 1 respectively, but with haulm yields of 5075 kg/ha and 3625 kg/ha, appear to share some characteristics. Looking at the data, we see that two lines from different populations had haulm yields above average, but very far apart on the graph, with pod yields of 1480 kg/ha and 1567.5 kg/ha being quite similar.

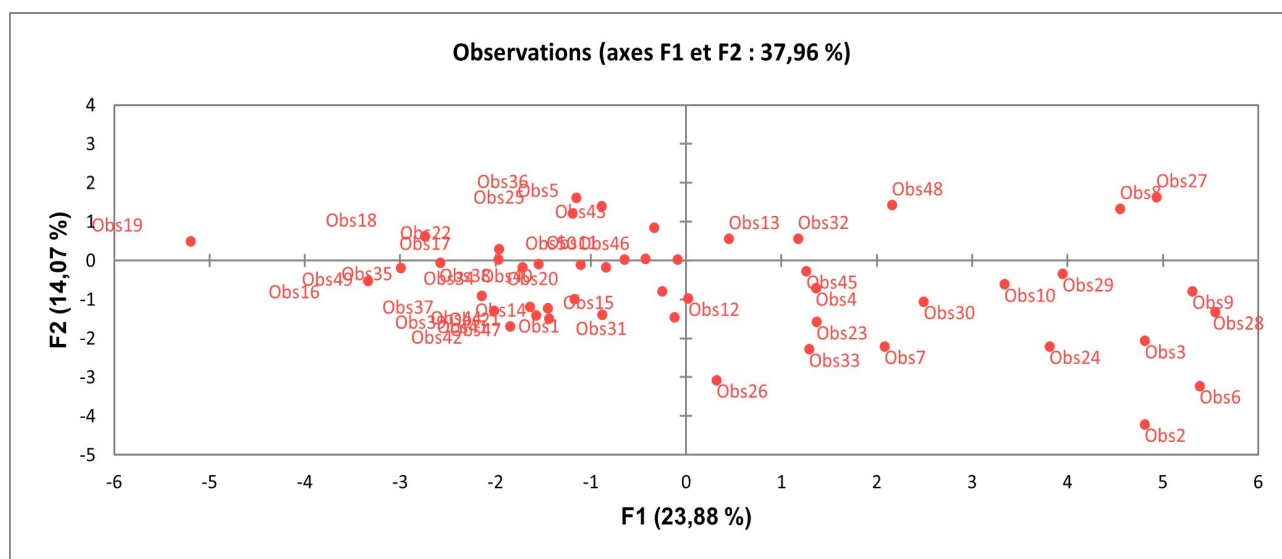


Figure 3. Scatter plot of the different treatments.

Hierarchical Ascending Classification (HAC)/Number of Classes = 2:

The dotted line represents the significance level relative to the truncation. Below this point, the dissimilarity is not high enough to significantly separate the data.

Here, the data has therefore been divided into two groups. The group on the right is less homogeneous than the group on the left because it has higher dissimilarity points. Inertia decomposition for the optimal classification (**Figure 3**)

Table 4. Decomposition of inertia for The optimal classification.

	Absolue	Pourcentage
Intra-classe	16052.900	82.26%
Interclasses	3460.865	17.74%
Inertie totale	19513.765	100.00%

Table 5. Results by class.

Classe	1	2
Nombre d'objets par classe	322	2
Somme des poids	322	2
Variance interclasse	48.746	405.483
Distance minimale au barycentre	0.371	14.239
Distance moyenne au barycentre	4.988	14.239
Distance maximale au barycentre	51.682	14.239
Objet	Obj1	Obj120
	Obj2	OBJ229
	Obj3	
	Obj4	
	objn	

Table 4 and **Table 5** shows the variance decomposition for the optimal classification: 82.26% is explained by the difference between classes, and 17.74% of the variance is explained by the remaining differences within classes. **Table 5** presents the list of treatments that constitute each class, along with the within-class variance and information regarding their positions relative to the centroid. Class (1) had a within-class variance of 48.746, while Class (2) had 405.483. As seen in (**Figure 3**), Class 1 is more homogeneous than Class 2. The treatments in Class 2 are, on average, further from its centroid than those in Class 1, with an average distance of 14.239 versus 4.988. The distance to the centroid of MLGV 211103 is 3,747 classes (1) and that of MLGV 2101032 is 14,239 classes (2). Using hierarchical ascending classification (HAC), the lineages were grouped into two classes that were as homogeneous as possible. However, one class is more homogeneous than the other, and a significant portion of the variance remains due to intra-class differences.

Dendrogram

This dendrogram (**Figure 4**) illustrates the results of a hierarchical classification of groundnut lines and cultivars based on their severity level in response to an artificial infection with the *C. personatum* isolate. The tree structure revealed significant heterogeneity in varietal responses to infestation, with mergers occurring at different levels of Euclidean distance. The identification of distinct classes, particularly at higher cut levels, allowed for the differentiation of highly resistant lines from those showing pronounced susceptibility. This cluster organization facilitated the strategic identification and selection of homogeneous genetic pools, essential for breeding programs aimed at pathogen resistance. Consequently, the structure of this dendrogram provided a robust empirical basis for line stratification and guided future crossings toward optimizing tolerance to *C. personatum*.

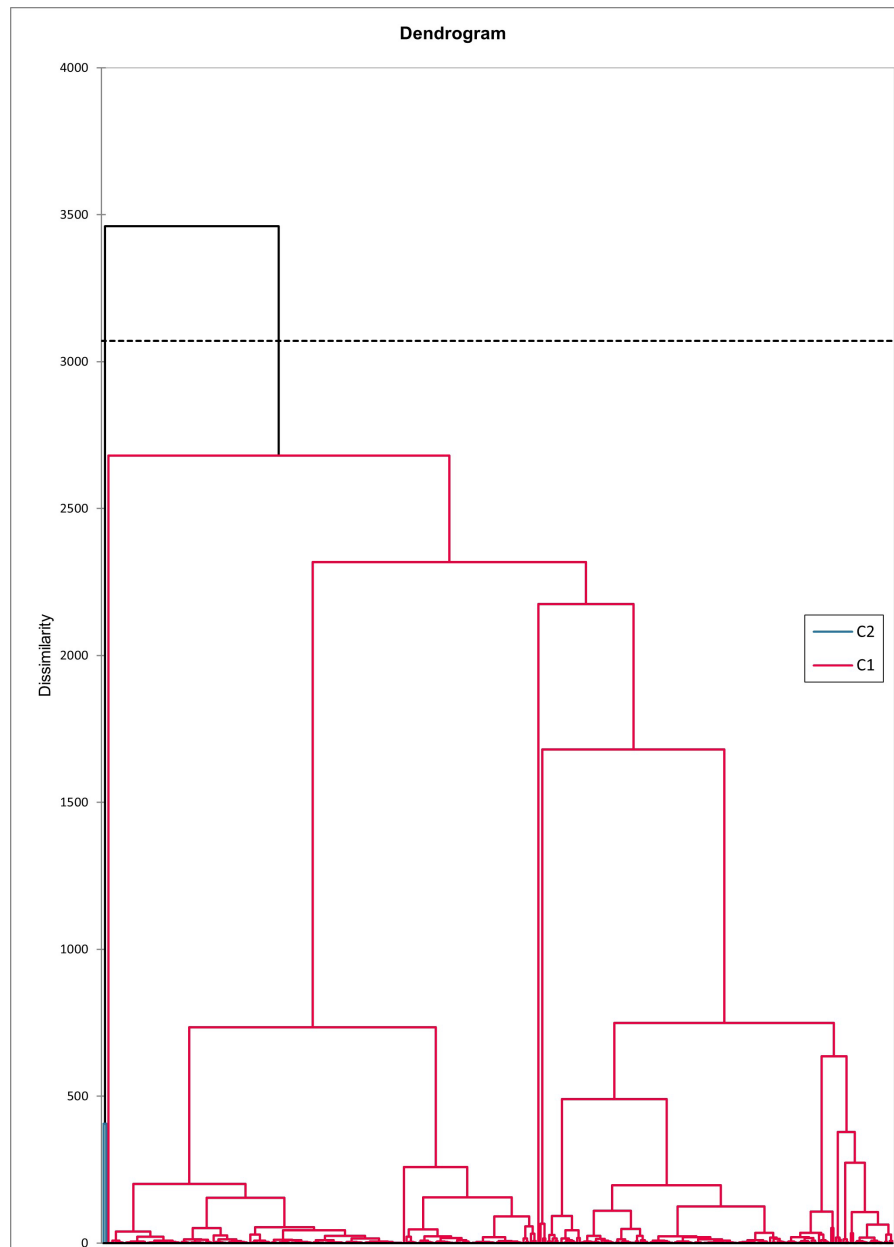


Figure 4. Dendrogram illustrating the severity level of 324 Groundnut lines and varieties subjected to artificial infection by a *C. personatum* isolate in a nursery (Ward's method).

3. Discussion

The initial natural contamination pressure of Groundnuts by cercospora leaf spot is quite high, given that the trial was established on a one-year fallow following Groundnut cultivation. Technical information showed that Groundnuts were frequently grown on the site. The initial fungal inoculum was preserved in the soil for several years. This value is very high with Groundnut monocultures or preceding susceptible crops, unlike cereal successions such as maize or sorghum [11]. This year's season (601 mm), with very high rainfall and well-distributed temperature and humidity conditions for harmonious fungal development and popula-

tion proliferation, should increase the level of infestation. Seed treatment was not applied to better preserve the initial fungal population in the soil. Although this practice does not protect the plant against disease, it allows for better germination and emergence performance and ensures the good health of the seedling.

Yields were below the performance shown by these lines in multi-location trials. The low yields could be explained by the fact that the soils were poor and much degraded, in part by the harsh conditions of setting up the trial during the off-season and the link to winter rains in July. One consequence of this was that maturation was abnormally prolonged, leading to pre-germination of the first mature pods because the lines are non-dormant, all descendants of Spanish varieties. Three hundred and twenty-four Groundnut lines were subjected to artificial inoculation in a nursery in 2024, with a local resistant control and a sub-regional resistant control. After artificial inoculation in the nursery, most varieties developed good resistance and the remainder moderately good resistance.

This variable reaction to the disease could be explained by the fact that the genetic makeup of each lineage and its ability to overcome the optimal extreme environmental conditions of the pathogen and its manifestations differ. The high broad-sense heritability calculated for key traits ($h^2 = 0.994$) confirms this predominant genetic basis and the potential effectiveness of selection.

The lines MLGV 2101141, MLGV 2102035, MLGV 2102046, MLGV 2102138, and MLGV 2101020 showed high resistance under artificial inoculation and moderate resistance in the field in 2023. With the best pod yields and good haulm yields, they demonstrated excellent performance. This performance, combined with high heritability, translates into a significant estimated genetic gain (G) potential per breeding cycle, on the order of 240 kg/ha for pods and 1.40 t/ha for haulm, highlighting the prospects for varietal improvement. In the 2022 – 2023 growing season, yields varied by region. This uneven distribution of yields provides a lot of information about the impact of the growing area on the expression of the potential of the lines [12].

Regarding sowing dates, flowering dates and 50% flowering, the production of fertile gynophores and the grouped maturity of pods guarantee good profitability [13]. Grouped flowering could ensure high fertilization, thus generating a high pod production [14]. In practice, early flowering promotes a good pod yield [15]. The early cessation of rainfall, pockets of drought, and poor soils partly explain the large variations in yield between areas [16]. These results agree with those of [17]-[19], who state that the damage caused by cercospora leaf spot results in a reduction of photosynthetic surface area due to the presence of numerous lesions and defoliation.

This damage leads to a deterioration in the organoleptic qualities of the seeds and low yields of haulm and pods. In Mali, Groundnut yields have fluctuated considerably in recent years due to climate change and various stresses. The degree of contamination and virulence of the pathogens responsible for foliar diseases, particularly cercospora leaf spot, have increased depending on the growing area. The

low yields observed in some varieties can be attributed to the lack of synchronization of flowering with periods favorable to fertilization. Consequently, most gynophores did not produce pods and/or were unable to develop into mature pods. Only early-flowering varieties are capable of producing a satisfactory yield under suitable conditions, hence the importance of selecting appropriate botanical types. Significant variations in disease severity and defoliation have been observed by zone (accounting for over 90% of the total annual variation) and by locality [20] [21]. The low rainfall in 2023, its very poor distribution, and the numerous pockets of drought could explain the low level of disease progression in the Samé and Kolokani agro-climatic zones, but the very severe disease in the Kita zone located in the Groundnut basin [22]. The significant interaction effects between genotypes and environments, which characterize the diverse behaviors of different genotypes, are consistent with the work of [23] and his team [24] and [21]. Significant interactions have been observed between varieties and environmental conditions, such as yield components and seed quality characteristics. Several studies demonstrate that artificial inoculation at different stages of Groundnut development or phenological phase's increases susceptibility to early cercospora leaf spot [20] [21]. However, some reservations remain: young plants inoculated 30 days after sowing show less disease, and contamination of new leaves remains negligible under controlled conditions for the following reasons [25]: Early cercospora leaf spot is a contact disease, not a systemic one. Its spread occurs through the deposition of conidia on leaves by wind, the splashing of raindrops on the ground, followed by spraying onto basal leaves, and insects [26]. These vectors are absent in controlled environments. The accumulation of phytoalexins in the plant organs blocks the infection of new leaves, as indicated by the work of Cole cited by [21] and [27] [28]. These authors demonstrated that leaves attacked early by *C arachidicola* produce phytoalexins (medicarpin), which are potent fungicides. Under these particular conditions, which are difficult for disease development and given that cercospora leaf spot is not a systemic disease, very young inoculated plants are only slightly affected until maturity. Conversely, plants inoculated at a later stage, when the leaves have fully emerged and have not yet produced a certain amount of phytoalexins [26], exhibit the most dramatic symptoms: a high severity of the disease with significant leaf drop in the following two or three weeks [29]. In Burkina Faso, the Nama and NCAc 17090 varieties showed greater tolerance to the disease in controlled environments, and these same varieties showed moderate resistance in several agro-climatic zones [21]. The research conducted shows that it is possible to determine Groundnut susceptibility under controlled conditions. This technique allowed for the screening of 324 Groundnut varieties against early cercospora leaf spot under controlled conditions in record time. Several genotypes showed moderate resistance and could aid future breeding programs.

4. Conclusion

It is safe to say that cercospora leaf spot is an extremely destructive disease for

groundnuts in Mali, affecting both haulm and pod yields. Yield reductions have been observed in all agro-climatic regions, particularly in the Sahel, with significant economic repercussions. To increase groundnut production and productivity in Mali, developing new lines resistant and/or tolerant to cercospora leaf spot will be beneficial in order to achieve future research objectives.

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Conflicts of interest

There are no conflicts of interest affecting the publication of this article.

Authors' Statement of Contributions

M.T., DS, D.H., and AD T. contributed to the design and planning of the study. M.T. performed the data collection and analysis. AD, M.K., KD, M.D., and NK. All authors have read and approved the submission of the manuscript for publication.

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