

Research Article

Genetics of Stay-Green Trait and Its Association with Leaf Spot Tolerance and Pod Yield in Groundnut

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Despite its importance in providing income and food for smallholder farmers, fodder for livestock, and improving soil fertility through biological nitrogen fixation, groundnut yields are lowest on farmers' fields in Sub-Saharan Africa due to biotic and abiotic constraints. Foliar fungal diseases account for over 80% reduction in groundnut productivity in some parts of Ghana. Unfortunately, chemical control of these foliar diseases has not yielded the desired results. Meanwhile, advances in phenotyping for disease tolerance in other crops have established a strong relationship between stay-green trait and foliar disease tolerance. However, this relationship has not been explored in groundnut. This study was designed to determine the genetic control of the stay-green trait and its relationship with leaf spot disease severity in groundnut. Twenty-five advanced groundnut breeding lines with varying degrees of tolerance for leaf spot tolerance were evaluated under diseased and disease-free conditions, after which four were selected for genetic studies. Results showed significant ($p < 0.001$) differences among the genotypes for early leaf spot (ELS), late leaf spot (LLS), leaf area under greenness (LAUG), SPAD chlorophyll meter readings (SCMR), and yield traits. Leaf spot diseases caused $4.95 \text{ t} \cdot \text{ha}^{-1}$ (64.54%) pod yield reduction in CHINESE, the widely cultivated groundnut variety in Ghana. There was a strong correlation between LAUG and ELS ($r = 0.82$, $p < 0.001$) and LLS ($r = 0.63$, $p < 0.001$), and genotypes that were stay-green had tolerance to both diseases. Stay-green trait in groundnut was detected to be under the control of a single recessive gene and hence may be used to select for ELS and LLS resistance.

1. Introduction

Groundnut (*Arachis hypogaea* L.) is an important leguminous crop that provides income and food for the poor smallholder farmers in developing countries [1]. The haulms after harvesting the pods are also used as nutritious fodder for livestock, especially during the dry season [2]. As a result, groundnut cultivation contributes to the sustainability of crop-livestock production systems in most African countries including Ghana. Although there has been a 9.2% expansion in the area under cultivation of groundnut since 2008 and the release of high-yielding cultivars [3], Sub-Saharan (SSA) Africa contributes only about 24% to the total global groundnut production [1].

Biotic and abiotic stresses have caused yield stagnation of groundnut in SSA affecting its total production [2]. Foliar fungal diseases are the major factors limiting groundnut productivity [4] with *Cercospora* leaf spot (CLS) being predominant [5]. In northern Ghana, CLS has been shown to cause up to 81.2% reduction in pod yield in groundnut [6]. The leaf spot diseases damage groundnut plants by reducing the available photosynthetic area through reduction of leaf area index, lesion formation, and stimulating leaflet abscission [7, 8]. As a result, it disrupts photosynthetic process which causes groundnut to produce less pods with inferior quality. The infections mainly attack the leaves leading to premature defoliation hence causing increase in stem percentage of the resulting fodder which reduces in vitro

digestibility [9] when the haulms are fed to animals. Leaf spot disease occurs in two forms; early leaf spot (ELS) which is caused by *Cercospora arachidicola* S. Hori (Berk and M. A. Curtis) and late leaf spot (LLS) caused by *Cercosporidium personatum* Deighton [10]. Early leaf spot is characterized by light brown spots surrounded by yellow halo, while spots of late leaf spot are black and usually without yellow halo [11]. ELS and LLS typically occur together, and their spread and severity are influenced by the field cropping history, temperature, and relative humidity [12]. The leaf spot disease epidemics are affected by weather patterns such as hot and wet conditions [13]. Temperatures in the range of 25 to 30°C and high relative humidity favour infection and disease development [14].

Efforts have been directed at chemical control of leaf spot diseases in northern Ghana [6]. However, it has only been partially effective in controlling the disease on farmers' fields [8] and substantially increases cost of production. The development and adoption of leaf spot resistant cultivars will therefore be a breakthrough to resource poor farmers in the middle- and low-income countries [15] including Ghana who cannot afford chemical control measures.

Genetic variation exists in cultivated groundnut for both ELS and LLS resistance, but the resistant genotypes are generally late maturing [16]. Direct selection for leaf spot resistance in groundnut is also difficult and has been reported to be associated with low yield, poor pod, and kernel characteristics and late maturity [16]. As a result, there is the need to consider other physiological traits that can confer tolerance to the two foliar diseases and enhance pod and haulm yields.

Stay-green is an important trait that allows plants to retain their leaves in an active photosynthetic state when exposed to stress conditions [17]. It has been found to be present in different crops [17] and widely used in breeding for disease resistance [18]. The stay-green trait can be scored on a large number of entries [19] and is associated with other important agronomic and physiological traits. It enables plants to maintain active photosynthesis under drought [20], enhances higher nitrogen concentration in plant organs [21], confers tolerance to drought, heat, cold, pathogens, and resistance to lodging [22, 23], and performs better under low nitrogen conditions [24]. Maintenance of green leaf area through grain filling has been associated with increased grain yield in wheat [25, 26], maize [27], and sorghum [28]. The stay-green trait occurs at three levels in plants, namely, the cell, leaf, and whole plant levels [29]. However, green leaf area at physiological maturity has proven to be a good measure of stay-green [30, 31] with the potential of enhancing foliar disease resistance in groundnuts.

The objectives of this study were to determine the (i) genetic control of the stay-green trait in groundnut and (ii) association between stay-green trait and leaf spots severity in groundnut.

2. Materials and Methods

2.1. Site, Plant Materials, and Design of Experiment. The experiment was carried out at the research station of Council

for Scientific and Industrial Research-Savanna Agricultural Research Institute (CSIR-SARI), Tamale, Ghana, located at 09° 25' 41" N, 00° 58' 42" W and altitude of 183 m above sea level. The study area is characterized by a relatively dry climate with a unimodal rainfall ranging between 900 and 1200 mm annually. The rainy season begins in May and ends in October with few scattered precipitation in November. The soils of the area are Ferric Luvisols of the Tingoli series [32, 33].

A total of 25 advanced breeding lines of groundnut with varied levels of tolerance to leaf spot diseases were selected from the germplasm collection at the CSIR-SARI (Table 1). These lines comprised of four (4) released varieties and twenty-one (21) advanced breeding lines assembled from ICRISAT EIR in Mali and INERA in Burkina Faso.

The study comprised of two parts: (i) evaluation of the genotypes for the stay-green trait and leaf spot (ELS and LLS) severity and (ii) genetic analysis of the selected genotypes for stay-green trait.

2.2. Evaluation of the Genotypes for the Stay-Green Trait and Tolerance to Leaf Spot Diseases. Evaluation of the 25 genotypes for leaf spot disease tolerance was done in the major cropping season when the disease incidence is usually highest. Disease infection was done under natural conditions with spreaders planted at border and between rows to ensure inoculum pressure for the disease development in 2016. To ensure zero incidence of the leaf spot diseases, the genotypes were evaluated for the stay-green trait in the minor season of 2016.

The experiment was laid in a partial lattice design with five blocks replicated three times. A plot comprised of four rows of 4 m long with inter- and intrarow spacing of 0.5 m and 0.1 m, respectively. One seed was planted per hill following the procedure of Naab et al. [7, 8]. The genotypes were grouped into late, medium, and early maturing based on the length of their maturity periods. Hence, planting dates were staggered in the order of late, medium, and early maturing group, respectively, to ensure that their reproductive stages (stage of high disease infection) coincided. Triple superphosphate (TSP) was applied at a rate of 60 kg P₂O₅ ha⁻¹ at two weeks after planting. The experiments were further supplemented with grounded oyster shells at a rate of 200 kg·ha⁻¹ to supply calcium [34].

2.3. Genetic Analysis of the Stay-Green Trait in Groundnut. After the field evaluation, two groundnut genotypes expressing the stay-green trait and resistance to leaf spot diseases (NKATIESARI and ICG 7878) were selected and crossed with two other genotypes, CHINESE and ICGV-IS 13081, that were non-stay-green and susceptible to leaf spot diseases using a biparental mating design. The F₁ progeny from each cross divided into two sets, one set was advanced to F₂, whereas the other set was saved for field evaluation.

The parents, F₁ and F₂, were evaluated for leaf spot severity and the stay-green trait during the major and minor seasons, respectively, in 2017. Spreaders were used to enhance the disease inoculum pressure on the disease

TABLE 1: Genotypes used in the study and their characteristics and sources where they were obtained.

Genotypes	Attributes	Source
NKATIESARI	Medium maturity, resistant to ELS and LLS	SARI, Ghana
YENYAWOSO	Early maturity, ELS and LLS susceptible	SARI, Ghana
KPANIELLI	Late maturity, ELS and LLS resistance	SARI, Ghana
CHINESE	Early maturity, susceptible to ELS and LLS	SARI, Ghana
GK 7	Late maturity, ELS and LLS resistance	IER-Burkina Faso
GAF 1665	Late maturity, ELS and LLS resistance	SARI, Ghana
GAF 1723	Late maturity, ELS and LLS resistance	SARI, Ghana
SUMNUT 23	Medium maturity, ELS and LLS tolerance	Nigeria
ICGV 00064	Late maturity, ELS and LLS resistance	ICRISAT, Mali
ICGV-IS 08837	Medium maturity, ELS and LLS tolerance	ICRISAT, Mali
ICGV-IS 13015	Medium maturity, foliar disease tolerance	ICRISAT, Mali
ICGV-IS 13018	Medium maturity, foliar disease tolerance	ICRISAT, Mali
ICGV-IS 13041	Medium maturity, foliar disease tolerance	ICRISAT, Mali
ICGV-IS 13045	Medium maturity, foliar disease tolerance	ICRISAT, Mali
ICGV-IS 13052	Medium maturity, foliar disease tolerance	ICRISAT, Mali
ICGV-IS 13071	Medium maturity, foliar disease tolerance	ICRISAT, Mali
ICGV-IS 13078	Medium maturity, foliar disease tolerance	ICRISAT, Mali
ICGV-IS 13079	Medium maturity, foliar disease tolerance	ICRISAT, Mali
ICGV-IS 13081	Medium maturity, foliar disease tolerance	ICRISAT, Mali
ICGV-IS 13086	Medium maturity, foliar disease tolerance	ICRISAT, Mali
ICGV-IS 13110	Medium maturity, foliar disease tolerance	ICRISAT, Mali
ICGV-IS 13113	Medium maturity, foliar disease tolerance	ICRISAT, Mali
ICGV-IS 13114	Medium maturity, foliar disease tolerance	ICRISAT, Mali
ICGV-IS 13998	Medium maturity, foliar disease tolerance	ICRISAT, Mali
ICG 7878	Late maturity, ELS and LLS resistance	ICRISAT, Mali

ELS: early leaf spot; LLS: late leaf spot.

evaluation plots. The experiment was arranged in a randomized complete block design (RCBD) with three replications. Single row plots of 2 m length were used with inter- and intrarow spacing kept at 0.5 m and 0.1 m, respectively.

2.4. Data Collection. Starting from pod initiation to physiological maturity visual, scoring for severity of ELS and LLS infection and stay-green were carried out at two weeks intervals. The severity of ELS and LLS infection was scored on a scale of 1 to 9 with 1 being completely resistant and 9 a dead plant [11]. The severity scores were used to compute the area under disease progress curve (AUDPC) value [35]. The stay-green characteristic of the genotypes was scored on a scale of 1 to 5 based on the proportion of the total leaf area that had senesced with 1 being no leaf senescence and 5 completely senesced plant [36]. The stay-green scores were used to compute the leaf area under greenness (LAUG) values [18]. Lower values of AUDPC and LAUG represent higher disease tolerance (lower disease severity) and stay-green in groundnut, respectively.

At pod initiation, data were also collected on leaf chlorophyll (SCMR) using a chlorophyll meter (TYS-A), and number of green leaves and leaf area (LA, cm²) using a leaf area meter (YMJ-B). In measuring SCMR, second fully opened leaves were clipped with the chlorophyll meter until readings stabilized and recorded. The leaves used to measure the SCMR were plucked for immediate leaf area measurement in the lab. Pod yield (PY, t·ha⁻¹) and reduction in pod yield (RPY, t·ha⁻¹) due to disease were also recorded at

maturity. All data were taken on five (5) randomly selected plants.

AUDPC, LAUG, and RPY were computed using the formulae below:

$$\text{AUDPC} = \sum_{i=1}^a \left[\left\{ \frac{Y_i + Y_{(i+1)}}{2} \right\} x (t_{(i+1)} - t_i) \right],$$

$$\text{LAUG} = \sum_{i=1}^a \left[\left\{ \frac{S_i + S_{(i+1)}}{2} \right\} x (t_{(i+1)} - t_i) \right], \quad (1)$$

$$\text{RPY} = \text{PY}_{\text{dfp}} - \text{PY}_{\text{dp}},$$

where Y_i is the disease level at time t_i , $t_{(i+1)} - t_i$ is the time in days between two sequential disease/stay-green scores, S_i is the visual score at time t_i , PY_{dfp} is the pod yield on disease-free plot, and PY_{dp} is the pod yield on disease plot.

2.5. Data Analysis. Statistical analysis was done using the R statistical software version 3.5.1 [37]. Using the lme4 package [38], a linear mixed effect model was fitted by the restricted maximum likelihood (REML) procedure to determine genotypic effects using the equation below:

$$Y_{ijk} = \mu + r_k + b_{jk} + g_i + \varepsilon_{ijk}, \quad (2)$$

where Y_{ijk} , μ , r_k , b_{jk} , g_i , and ε_{ijk} , denote the observation on genotype i in block j of replication k , grand mean, effect of replication k , effect of block j nested within replication k , effect of genotype i , and the residual effect, respectively.

Replicates, blocks nested within replicates, and the residual effect were all considered as random effects and genotypes as fixed effect. Fixed effect estimates were compared using *t*-tests based on Satterthwaite's method for correcting degrees of freedom for Levene's test of homogeneity of variance [39]. Effect comparison was done using the lmerTest package [40]. CHINESE, which is the most cultivated groundnut variety in Ghana's guinea savanna agroecology, was set as the intercept using the "relevel()" command of R.

Based on the scale used in scoring stay-greenness, if a genotype has LAUG score of between 1 and 28, it was regarded as stay-green, between 29 and 42 as moderately stay-green, while between 43 and 70 as non-stay-green [36]. The genotypes were assigned to the various stay-green classes (stay-green, moderately stay-green, and non-stay-green) using the LAUG scores. The different classes were compared using variance component analysis and the comparison was done with the agricolae package [41] of R. To confirm the influence of stay-greenness on leaf spot tolerance, principal component analysis (PCA) was computed for the AUDPC scores of ELS and LLS using the vegan package [42] of the R statistical software.

Coefficients of relationship between pod yield, LAUG, SCMR, LA, ELS, and LLS AUDPC were estimated based on Pearson's correlation criteria, while boxplot analysis was used to plot mean leaf chlorophyll content of the genotypes against growth stage to understand the variation of progress of leaf chlorophyll degradation among genotypes till the reproductive stage.

For the genetic analysis, the chi-square test was used to determine if the ratios of segregations observed at F_2 fitted expected Mendelian ratios. Broad and narrow sense heritability were estimated using the variances from biparental mating design (BIP) of parents and offsprings [43] (Table 2):

$$\delta_b^2 = \frac{1}{2}V_A + \frac{1}{4}V_D + V_{EC} = \frac{1}{r}(MS_1 - MS_2), \quad (3)$$

$$\delta_w^2 = \frac{1}{2}V_A + \frac{3}{4}V_D + V_{EW} = MS_2.$$

Narrow and broad sense heritabilities were estimated as shown in the following equations [44]:

$$\frac{\text{covariance of offspring}}{\text{midparent value}} = \frac{1}{2}V_A,$$

$$h^2 = \frac{V_A}{V_A + V_D + V_{EW}}, \quad (4)$$

$$H^2 = \frac{V_D}{V_A + V_D + V_{EW}},$$

where h^2 is the narrow sense heritability, H^2 is the broad sense heritability, V_A is the additive variance, V_D is the dominance variance, V_{EC} is the environmental variance among families, and V_{EW} is the environmental variance within families.

TABLE 2: Variance components for biparental mating design.

Sources of variation	df	Ms	EMS
Between families	$(1/2n) - 1$	MS_1	$\delta_b^2 + r\delta_w^2$
Within families	$(1/2n)(r - 1)$	MS_2	δ_w^2
Total	$(1/2n) - 1$	—	—

n : number of parents sampled per cross; r : number of individuals sampled per cross; δ_b^2 : between family variance; δ_w^2 : within family variance; MS_1 : between family mean square; MS_2 : within family mean square.

3. Results

3.1. Variation of Area under Disease Progress Curve for Leaf Spots and Leaf Area under Greenness among Groundnut Genotypes. The genotypes evaluated differed significantly ($p < 0.001$) for leaf spot disease incidence and severity (Table 3). CHINESE had the highest AUDPC for ELS, but the severity was similar to that of ICGV-IS 13081, ICGV-IS 08837, ICGV-IS 13113, ICGV-IS 13114, ICGV-IS 13110, ICGV-IS 13018, ICGV-IS 13078, ICGV-IS 13041, and SUMNUT 23, while NKATIESARI expressed the lowest AUDPC for ELS (Table 3).

SUMNUT 23 had the highest AUDPC for LLS but was similar ($p \geq 0.05$) to that of CHINESE (Table 3). The score of CHINESE, however, did not differ statistically for LLS from that of ICGV-IS 13113, ICGV-IS 13110, ICGV-IS 08837, ICGV-IS 13041, ICGV-IS 13114, ICGV-IS 13018, and ICGV-IS 13081. The least AUDPC for LLS was expressed by ICG 7878 and GAF 1665.

Furthermore, CHINESE had the highest leaf area under greenness (LAUG) (58.54, $p < 0.001$) followed by ICGV-IS 08837, while NKATIESARI had the lowest LAUG (Table 3). Among the 25 genotypes tested, nine were categorized as stay-green, 12 moderately stay-green, and only four as non-stay-green (Table 3). The stay-green genotypes consisted of NKATIESARI, ICG 7878, GAF 1665, GAF 1723, GK 7, ICGV 00064, ICGV-IS 13114, ICGV-IS 13998, and KPANIELLI; the moderately stay-green genotypes were ICGV-IS 13015, ICGV-IS 13018, ICGV-IS 13041, ICGV-IS 13045, ICGV-IS 13052, ICGV-IS 13071, ICGV-IS 13078, ICGV-IS 13079, ICGV-IS 13086, ICGV-IS 13110, ICGV-IS 13113, and YENYAWOSO; while CHINESE, ICGV-IS 13081, ICGV-IS 08837, and SUMNUT 23 were considered as non-stay-green (Table 3).

3.2. Chlorophyll Content, Leaf Area (LA), Leaf Number, Pod Yield, and Reduction in Pod Yield due to Disease of Groundnut. Leaf chlorophyll content measured on genotypes under disease-free conditions at pod initiation and midpod filling was highest ($p < 0.01$) in NKATIESARI and GAF 1665, compared to CHINESE (Table 4). CHINESE had lower leaf chlorophyll than GAF 1723, KPANIELLI, NKATIESARI, and SUMNUT 23 at pod initiation, while KPANIELLI, NKATIESARI, and SUMNUT 23 had lower leaf chlorophyll content at midpod filling (Table 4). At physiological maturity, GK 7 had significantly ($p < 0.05$) higher leaf chlorophyll content, while ICGV-IS 08837 had lower leaf chlorophyll than CHINESE, respectively.

TABLE 3: Area under disease progress curve for early leaf spot (ELS AUDPC) and late leaf spot (LLS AUDPC) diseases and leaf area under greenness (LAUG) of 25 groundnut genotypes evaluated under disease and disease-free conditions in 2017.

Genotypes	ELS AUDPC			LLS AUDPC			LAUG		
	Estimate	Std. error	Sig	Estimate	Std. error	Sig	Estimate	Std. error	Sig
CHINESE (intercept)	215.80	11.71	***	205.01	16.54	***	58.54†	3.41	***
GAF 1665	-96.61	16.56	***	-85.22	20.68	***	-40.43§	4.17	***
GAF 1723	-88.57	16.48	***	-63.45	20.31	**	-35.69§	4.17	***
GK 7	-57.81	16.56	**	-30.56	20.68	ns	-38.02§	4.17	***
ICG 7878	-91.11	16.55	***	-82.23	20.66	***	-34.32§	4.14	***
ICGV-IS 00064	-73.68	16.56	***	-50.57	20.71	*	-39.74§	4.14	***
ICGV-IS 08837	-8.20	16.56	ns	-12.04	20.70	ns	-11.39†	4.14	***
ICGV-IS 13015	-56.44	16.56	**	-46.67	20.71	*	-24.16‡	4.17	***
ICGV-IS 13018	-23.36	16.48	ns	-12.13	20.29	ns	-22.17‡	4.14	***
ICGV-IS 13041	-24.67	16.56	ns	-14.66	20.68	ns	-19.97‡	4.17	***
ICGV-IS 13045	-42.71	16.48	*	-65.84	20.30	**	-20.59‡	4.17	***
ICGV-IS 13052	-40.05	16.48	*	-50.20	20.27	*	-22.10‡	4.17	***
ICGV-IS 13071	-37.77	16.41	*	-49.38	19.93	*	-18.94‡	4.14	***
ICGV-IS 13078	-32.48	16.55	ns	-43.89	20.67	*	-22.86‡	4.17	***
ICGV-IS 13079	-33.39	16.48	*	-46.41	20.29	*	-22.65‡	4.14	***
ICGV-IS 13081	-1.71	16.56	ns	-12.23	20.69	ns	-14.28†	4.14	***
ICGV-IS 13086	-41.75	16.41	*	-50.29	19.93	*	-26.01‡	4.14	***
ICGV-IS 13110	-21.57	16.56	ns	-12.70	20.68	ns	-24.91‡	4.17	***
ICGV-IS 13113	-17.95	16.55	ns	-5.11	20.65	ns	-25.81‡	4.17	***
ICGV-IS 13114	-16.82	16.55	ns	-12.25	20.66	ns	-29.58§	4.14	***
ICGV-IS 13998	-48.33	16.49	**	-46.12	20.34	*	-32.53§	4.14	***
KPANI ELI	-69.80	16.49	***	-50.55	20.32	*	-35.14§	4.14	***
NKATIESARI	-98.33	16.49	***	-81.12	20.34	***	-40.77§	4.17	***
SUMNUT 23	-27.08	16.55	ns	9.59	20.67	ns	-14.00†	4.14	***
YENYAWOSO	-42.18	16.56	*	-63.81	20.69	**	-17.98‡	4.17	***

*, **, and *** indicate significance at 0.05, 0.01, and 0.001 levels of probability, respectively. ns: not significant. LLS: late leaf spot; ELS: early leaf spot; AUDPC: area under disease progress curve; LAUG: stay-green trait. § indicates stay-green genotype. ‡ indicates moderately stay-green genotype. † indicates non-stay-green genotype.

Under disease conditions, GK 7 had the highest leaf chlorophyll content at pod initiation which was significantly ($p \leq 0.01$) different from that of CHINESE (Table 5). Also, GAF 1665, GAF 1723, and SUMNUT 23 all had higher leaf chlorophyll than CHINESE. At midpod filling, all the genotypes were similar in terms leaf chlorophyll content except ICGV-IS 13052 which had significantly ($p < 0.05$) lower chlorophyll content than that of CHINESE (Table 5). At physiological maturity, NKATIESARI had the highest leaf chlorophyll content under disease conditions which was significantly ($p \leq 0.01$) different from that of CHINESE. GAF 1665, GK 7, ICG 7878, ICGV-IS 13015, ICGV-IS 13113, and KPANI ELI had higher leaf chlorophyll content than that of CHINESE (Table 5).

The total number of green leaves per plant varied ($p < 0.01$) among the genotypes. GAF 1665, GAF 1723, GK 7, ICG 7878, ICGV-IS 00064, KPANI ELI, and NKATIESARI had significantly ($p < 0.01 \leq 0.001$) higher number of green leaves than CHINESE at pod initiation (Table S1). At physiological maturity, CHINESE had similar number of green leaves compared to most genotypes. However, ICG 7878, NKATIESARI, KPANI ELI, ICGV-IS 13114, ICGV-IS 13045, ICGV-IS 13015, ICGV-IS 00064, GAF 1665, GAF 1723, and GK 7 had significantly ($p < 0.05 \leq 0.001$) higher number of green leaves.

Under disease-free conditions, genotypes YENYAWOSO and CHINESE had the highest number of leaves at pod initiation and physiological maturity, respectively

(Table S2). On the contrary, ICGV-IS 13015, ICGV-IS 13071, ICGV-IS 13079, ICGV-IS 13086, and ICGV-IS 13113 had lower green leaf number than CHINESE at pod initiation. At physiological maturity, ICGV-IS 13045 and ICGV-IS 13114 were the only two genotypes with similar number of leaves ($p \geq 0.05$) as CHINESE. The rest of the genotypes had significantly ($p < 0.05 \leq 0.001$) lower leaf number than that of CHINESE (Table S2). Furthermore, genotype CHINESE had the highest leaf area (LA) at pod initiation but similar to that of ICGV-IS 13081, ICGV-IS 13086, ICGV-IS 13018, ICGV-IS 13041, ICGV-IS 13052, ICGV-IS 13110, ICGV-IS 13114, ICGV-IS 08837, ICGV-IS 13113, and ICGV-IS 13079 (Table S2). However, it had a leaf area which was higher than that of all the other genotypes including ICG 7878 and NKATIESARI.

When plants were infected by the leaf spot diseases, CHINESE recorded the least pod yield of 2.23 t·ha⁻¹ while NKATIESARI gave 6.18 t·ha⁻¹ as the highest (Table S3). Under disease-free conditions, CHINESE and NKATIESARI gave similar pod yields of about 7 t·ha⁻¹. The reduction in pod yield due to disease was highest in CHINESE (4.95 t·ha⁻¹) which was about 64.54% of pod yield obtained under disease-free conditions (Table S3).

3.3. Relationship between the Stay-Green Trait (LAUG), Leaf Spot Severity (AUDPC), Leaf Chlorophyll Content (SCMR),

TABLE 4: Leaf chlorophyll content (SCMR) of 25 groundnut genotypes evaluated under disease-free conditions.

Genotypes	SCMR at pod initiation			SCMR at midpod filling			SCMR at physiological maturity		
	Estimate	Std. error	Sig	Estimate	Std. error	Sig	Estimate	Std. error	Sig
CHINESE (intercept)	26.95	1.70	***	28.74	1.94	***	32.68	2.28	***
GAF 1665	8.00	2.32	**	7.22	2.24	**	1.80	2.33	ns
GAF 1723	5.06	2.32	*	3.31	2.24	ns	2.45	2.33	ns
GK 7	4.25	2.32	ns	1.45	2.24	ns	5.68	2.33	*
ICG 7878	2.53	2.26	ns	0.83	2.18	ns	2.11	2.27	ns
ICGV-IS 00064	1.39	2.26	ns	-0.04	2.18	ns	0.28	2.27	ns
ICGV-IS 08837	0.49	2.26	ns	-2.39	2.18	ns	-4.93	2.27	*
ICGV-IS 13015	4.54	2.32	ns	3.45	2.24	ns	1.76	2.33	ns
ICGV-IS 13018	-0.74	2.26	ns	-1.45	2.18	ns	-0.13	2.27	ns
ICGV-IS 13041	1.28	2.32	ns	-2.89	2.24	ns	-3.28	2.33	ns
ICGV-IS 13045	0.42	2.32	ns	0.36	2.24	ns	-0.55	2.33	ns
ICGV-IS 13052	2.79	2.32	ns	0.29	2.24	ns	-4.35	2.33	ns
ICGV-IS 13071	2.88	2.26	ns	0.59	2.18	ns	0.12	2.27	ns
ICGV-IS 13078	0.71	2.32	ns	3.58	2.24	ns	0.18	2.33	ns
ICGV-IS 13079	2.14	2.26	ns	0.87	2.18	ns	-0.27	2.27	ns
ICGV-IS 13081	3.44	2.26	ns	2.32	2.18	ns	1.54	2.27	ns
ICGV-IS 13086	1.72	2.26	ns	-2.51	2.18	ns	-2.21	2.27	ns
ICGV-IS 13110	4.50	2.32	ns	1.57	2.24	ns	-0.21	2.33	ns
ICGV-IS 13113	4.86	2.32	*	-0.09	2.24	ns	-1.11	2.33	ns
ICGV-IS 13114	2.95	2.26	ns	0.11	2.18	ns	1.15	2.27	ns
ICGV-IS 13998	1.69	2.26	ns	-0.52	2.18	ns	-3.28	2.27	ns
KPANIELLI	6.52	2.26	**	5.25	2.18	*	3.78	2.27	ns
NKATIESARI	8.21	2.32	***	4.74	2.24	*	2.42	2.33	ns
SUMNUT 23	6.22	2.26	**	5.71	2.18	*	2.57	2.27	ns
YENYAWOSO	3.71	2.32	ns	1.93	2.24	ns	-2.32	2.33	ns

*, **, and *** indicate significance at 0.05, 0.01, and 0.001 levels of probability, respectively. ns: not significant. SCMR: leaf chlorophyll content.

TABLE 5: Leaf chlorophyll content (SCMR) of 25 groundnut genotypes evaluated under disease conditions.

Genotypes	SCMR at pod initiation			SCMR at midpod filling			SCMR at physiological maturity		
	Estimate	Std. error	Sig	Estimate	Std. error	Sig	Estimate	Std. error	Sig
CHINESE (intercept)	26.40	1.515	***	26.34	1.690	***	26.38	2.601	***
GAF 1665	3.78	1.672	*	2.80	2.361	ns	5.12	2.080	*
GAF 1723	4.11	1.616	*	0.90	2.298	ns	3.72	2.029	ns
GK 7	5.19	1.675	**	1.62	2.363	ns	4.19	2.081	*
ICG 7878	1.42	1.667	ns	-0.81	2.356	ns	4.70	2.076	*
ICGV-IS 00064	3.03	1.680	ns	1.30	2.368	ns	2.19	2.085	ns
ICGV-IS 08837	-2.23	1.677	ns	-3.69	2.366	ns	-2.19	2.083	ns
ICGV-IS 13015	1.66	1.682	ns	-1.75	2.369	ns	4.24	2.086	*
ICGV-IS 13018	1.13	1.611	ns	-3.68	2.294	ns	1.40	2.026	ns
ICGV-IS 13041	0.42	1.674	ns	-3.32	2.363	ns	0.42	2.081	ns
ICGV-IS 13045	1.56	1.613	ns	-3.98	2.295	ns	2.48	2.027	ns
ICGV-IS 13052	-0.45	1.605	ns	-4.78	2.288	*	-2.71	2.022	ns
ICGV-IS 13071	0.01	1.558	ns	-2.08	2.233	ns	3.06	1.977	ns
ICGV-IS 13078	-1.92	1.671	ns	-3.25	2.360	ns	-0.14	2.079	ns
ICGV-IS 13079	1.18	1.612	ns	-1.52	2.294	ns	2.56	2.026	ns
ICGV-IS 13081	-1.29	1.676	ns	-1.93	2.365	ns	1.67	2.083	ns
ICGV-IS 13086	0.64	1.557	ns	-4.35	2.232	ns	1.01	1.977	ns
ICGV-IS 13110	1.29	1.674	ns	-3.60	2.363	ns	1.25	2.081	ns
ICGV-IS 13113	1.38	1.666	ns	-0.68	2.355	ns	4.24	2.076	*
ICGV-IS 13114	-1.18	1.666	ns	-2.37	2.356	ns	0.45	2.076	ns
ICGV-IS 13998	-0.17	1.626	ns	-3.14	2.306	ns	-0.48	2.035	ns
KPANIELI	1.11	1.622	ns	-0.41	2.303	ns	4.95	2.032	*
NKATIESARI	3.07	1.626	ns	2.67	2.306	ns	6.22	2.035	**
SUMNUT 23	4.09	1.671	*	-1.64	2.360	ns	0.97	2.079	ns
YENYAWOSO	1.62	1.675	ns	-1.94	2.364	ns	0.29	2.082	ns

*, **, and *** indicate significance at 0.05, 0.01, and 0.001 levels of probability, respectively. ns: not significant. SCMR: leaf chlorophyll content.

Leaf Area (LA), and Pod Yield. There were significant correlations among all the traits considered (Table 6). Pod yield had negative correlation with LAUG ($r = -0.68$, $p < 0.001$), ELS AUDPC ($r = -0.62$, $p < 0.01$), LLS AUDPC ($r = -0.41$, $p < 0.05$), and LA ($r = -0.68$, $p < 0.001$). Pod yield, however, had strong and positive correlation with SCMR ($r = 0.70$, $p < 0.001$) at pod initiation, midpod filling ($r = 0.68$, $p < 0.001$) and physiological maturity ($r = 0.58$, $p < 0.01$).

On the contrary, LAUG had strong and positive associations with LA at pod initiation ($r = 0.63$, $p < 0.001$), ELS ($r = 0.82$, $p < 0.001$), and LLS ($r = 0.63$, $p < 0.001$) AUDPCs (Table 6). Also, LA at pod initiation had positive correlations with ELS ($r = 0.79$, $p < 0.001$) and LLS ($r = 0.54$, $p < 0.01$) AUDPCs.

Results from the principal component analysis conducted using the AUDPCs of ELS and LLS gave clusters of genotypes that were similar to the stay-green (LAUG) classes observed in Table 3 (Figure 1). Cluster "A" was made up of genotypes that had stay-green except ICGV-IS 13015 which expressed moderately stay-green based on the LAUG procedure. Clusters "B" and "C" were made up of genotypes with moderately stay-green, while cluster "D" consisted of non-stay-green genotypes (Figure 1).

Using LAUG as a stay-green criterion to group the germplasm, nine genotypes were found to be stay-grain (SG), twelve moderately stay-green (MSG), and four non-stay-green (NSG) (Tables 3 and 7). Variance component analysis of the stay-green classes showed significant ($p < 0.01$) differences among the genotypes for LAUG, ELS AUDPC, LLS AUDPC, and pod yield. The stay-green class had the lowest LAUG, ELS AUDPC, LLS AUDPC, and the highest pod yield (Table 7), while the non-stay-green class recorded the highest LAUG, ELS AUDPC, LLS AUDPC, and the lowest pod yield. The moderately stay-green class of genotypes was in between the two extreme classes (Table 7).

3.4. Genetic Analysis of the Stay-Green Trait

3.4.1. LAUG of Parents and F_1 Generations, Segregation Ratios at F_2 , and Heritability Estimates of LAUG in the Population. To understand the genetic basis of the stay-green trait, two stay-green and two non-stay-green genotypes were crossed for further analysis. Results of field evaluation of the parents together with the progeny showed that CHINESE and ICGV-IS 13081 non-stay-green parents had significantly ($p < 0.01$) the highest mean LAUG compared to that of NKATIESARI and ICG 7878 (Table 8). F_1 from ICGV-IS 13081 \times NKATIESARI and CHINESE \times ICG 7878 crosses designated as SARGV 007 and SARGV 006, respectively, differed statistically from either parents and among themselves. Based on the stay-green classification criteria, SARGV 006 was classified as stay-green, while SARGV 007 as moderately stay-green (Table 8).

The phenotypic variance was partitioned into the various components (Table 8) to estimate progress that can be realized through breeding. The genetic component of the variation (18.98) was higher than that of the environment (3.17). Dominance was significantly higher (16.94) than the

additive (2.04) genetic variances (Table 8). Heritability estimates ranged from a low narrow sense heritability (h^2 , 0.09) to a very high broad sense heritability (H^2 , 0.86).

Generation mean analysis of selected F_1 progeny advanced to F_2 and evaluated for the stay-green trait showed that there was no significant difference ($p > 0.05$) between observed and expected ratios at F_2 based on the chi-square test (Table 9). The F_2 population segregated in the ratio of 1 (stay-green) : 3 (non-stay-green).

4. Discussion

Biotic stresses such as disease infection reduce groundnut yield in many environments. Despite considerable efforts directed at fungicidal control of the disease in the guinea Savanna zone of Ghana [6], there is high incidence of leaf spot diseases on farmers' fields largely due to partial effectiveness of chemical control measures [8]. As a result, sustained maintenance of genetic progress in the improvement of disease resistance is essential, and this will require the identification and combination of new genetic variation. The AUDPC is a very convenient summary of plant disease epidemics that incorporates initial intensity, the rate parameter, and the duration of the epidemic which determines final disease intensity [45]. Based on the leaf spot severity scores observed in this study, genotypes GAF 1665, GAF 1723, ICG 7878, and NKATIESARI were highly resistant to early and late leaf spot diseases. Also, stay-green (SG), moderately stay-green (MSG), and non-stay-green (NSG) genotype classes were found to have significant AUDPCs for ELS and LLS with SG class having the least values. This result depicts the resistance of the genotypes to leaf spot diseases. The high level of leaf spot tolerance exhibited by the SG class might be due to their leaf characteristics such as thick palisade layer, dark green colour, and small stomata which have all been found to obstruct the growth of spores of *Cercospora arachidicola* and *Cercosporidium personatum* [46]. It is therefore not surprising that leaf chlorophyll content at pod initiation, midpod filling and physiological maturity all had strong negative correlation with the AUDPCs of ELS and LLS conferring resistance to the pathogens.

Stay-greenness based on visual score of the proportion of green leaf area maintained at physiological maturity has been used as a criterion for selecting stay-green genotypes in maize [19], sorghum [36], wheat [18, 47], and sunflower [48]. The significant differences observed among the groundnut genotypes for LAUG in this study was due to the varied degrees of leaf senescence at physiological maturity during which the stay-green trait was expressed. The observed genotypic difference in the expression of the trait can be attributed to the different genetic backgrounds of the genotypes. Borrell et al. [28] that the stay-green trait is associated with Stg QTL regulating canopy size through constraining the size of the upper leaves. Genotypes that exhibited lower rates of leaf senescence through physiological maturity (lower LAUG) were also observed to have relatively smaller leaf area. Similar clustering patterns were also observed when the genotypes were grouped based on

TABLE 8: Leaf area under greenness of parents, F_1 s, and estimates of genetic variance components and heritability for stay-green trait.

Means genotypes	Mean LAUG	Variance components and heritability	
		Genetic parameter	LAUG
CHINESE	42.00 ± 1.16 ^a	Phenotypic variance (V_p)	22.16
ICGV-IS 13081	42.00 ± 0.01 ^a	Environmental variance (V_E)	3.17
SARGV 007 (F_1)	32.67 ± 0.73 ^b	Genetic variance (V_G)	18.98
SARGV 006 (F_1)	28.00 ± 1.04 ^c	Additive variance (V_A)	2.04
NKATIESARI	11.67 ± 1.17 ^d	Dominance variance (V_D)	16.94
ICG 7878	10.50 ± 0.01 ^d	Broad sense heritability (H^2)	0.86
Midparent value	26.54	Narrow sense heritability (h^2)	0.09

Means followed by same letters are not statistically different.

TABLE 9: Proportion of stay-green classes (segregation ratios) at F_2 .

Genotype	Observed		Expected		Total	χ^2 (1:3)	p value
	Stay-green	Non-stay-green	Stay-green	Non-stay-green			
SARGV 006-1	2	8	2.5	7.5	10	0.133	0.715
SARGV 008-1	3	10	3.25	9.75	13	0.026	0.873

χ^2 : chi-square.

replacing leaves which have been lost through natural senescence. However, in the presence of leaf spot disease infections, CHINESE loses this ability resulting in a reduced number of leaves at physiological maturity compared to pod initiation stage.

Crop maturity is usually characterized by senescence, chlorophyll loss, and a progressive decline in photosynthetic capacity. However, some genotypes exhibit decreased or delayed chlorophyll catabolism [49] resulting in maintenance of their chlorophyll content for a longer period. This phenomenon was portrayed in the groundnut population used in this study. During the reproductive phase, genotypes that had lower LAUG also had relatively higher mean chlorophyll content at pod initiation, mid-pod filling, and physiological maturity. Although there was no significant difference among the genotypes for leaf chlorophyll content during the reproductive phase, variation among the genotypes increased as the crops advanced towards physiological maturity with midpod filling being the stage with the highest variance (results not shown). This indicates that some genotypes (non-stay-green) were undergoing rapid leaf chlorophyll catabolism than others (stay-green), and hence, the lack of non-constant variance across the reproductive stage. These results suggest that the most important stay-green classes (stay-green and non-stay-green) required to make breeding progress are present in the population.

However, the genotypes performed differently under the disease and disease-free conditions in pod yield with CHINESE being the worst affected under the disease conditions but became the best when there was no leaf spot disease infection. The percent reduction in pod yield due to leaf spot diseases in CHINESE was about 65%. This highlights the importance of leaf spot diseases as a major yield reduction stress in groundnut production [4, 5].

The strong positive relationship between LAUG and AUDPC for ELS and LLS suggest the possible influence of

the stay-green trait on leaf spot severity. Also, the ability of the AUDPC scores to segregate the genotypes into groups synonymous to those of LAUG further highlights the interplay of these three traits in groundnut. The results agree with findings of different authors [49, 50] who showed that stay-green trait influences different diseases in other crops. For instance, sorghum recombinant inbred lines (RILs) carrying stay-green QTLs (stg1, stg3, or both (stg1 + 3)) expressed high levels of resistance to *Macrophomina phaseolina* and *Fusarium thapsinum* [50]. Also, the negative association between LAUG and pod yield means selecting for stay-greenness (lower LAUG) will enhance pod yield. This may be achieved either through reduced disease severity or enhanced photosynthetic activity of the plant via delay in chlorophyll catabolism [49], which results in prolonging the active photosynthetic period. The strong association between LAUG, pod yield, and ELS and LLS AUDPC with leaf chlorophyll content suggests that sustained leaf chlorophyll content during the reproductive phase is essential for maintenance of stay-greenness, higher pod yield, and higher tolerance to leaf spot diseases in groundnut.

The observed significant difference between the stay-green and non-stay-green parents for LAUG suggests that genetic differences existed between the parental lines used for the crosses. The LAUG of the F_1 generation was higher than the midparent value and were closer to that of the non-stay-green parents. It is therefore possible that the allele controlling the non-stay-green trait is partially dominant over the stay-green allele [43]. The 1:3 segregation ratio of stay-green to non-stay-green genotypes observed at F_2 also indicates stay-greenness in groundnut is controlled qualitatively by a single recessive gene. The trait can therefore be expressed only when the two alleles are in the same state. Similar findings have been reported in wheat [51] and rice [52]. However, in maize, it has been reported that the stay-green trait is quantitatively inherited with 17 QTLs clustered on four chromosomes accounting

for 73.08% of the total genetic variance [53]. The higher broad sense heritability compared to the narrow sense heritability observed for stay-green trait in the current study implies that making selection from the existing population will bring about more genetic advancement than making crosses and selecting from the progenies [44].

5. Conclusion

The stay-green trait (LAUG) had a positive association with ELS and LLS and, hence, has the ability to confer resistance to leaf spot disease in groundnut. Since it is relatively easier to score for this trait on a large number of entries, groundnut breeders can use this trait to select for ELS and LLS resistance in groundnut in areas with low disease pressure. Leaf area at pod initiation and leaf chlorophyll at pod initiation, midpod filling, and at physiological maturity can also be used to select for stay-green, ELS, and LLS resistance since there was a strong association between them. Our results show that stay-green trait in groundnut is under the control of a single recessive gene. Since the genotypes used were advanced breeding lines, which have attained homozygosity at majority of their loci, making selection from the existing population will bring about more genetic gain due to the high broad sense heritability.

Data Availability

Data used to support these findings and the analysis code can be sourced from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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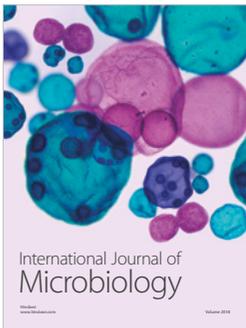
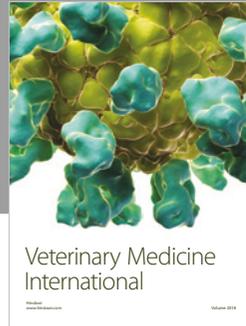
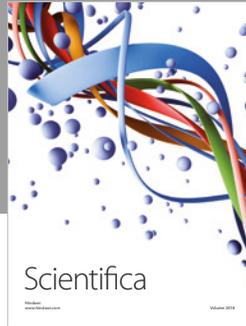
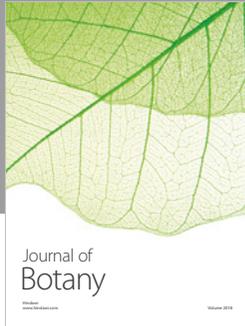
Supplementary Materials

Table S1: number of green leaves (NGL) of 25 groundnut genotypes evaluated under disease conditions. Table S2: leaf area (LA) and number of green leaves (NGL) of 25 groundnut genotypes evaluated under disease-free conditions. Table S3: pod yield and its reduction due to ELS-LLS infection of 25 groundnut genotypes evaluated under disease and disease-free conditions. (*Supplementary Materials*)

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