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EVALUATION OF GROUNDNUT (*Arachis hypogaea* L.) ADVANCED BREEDING LINES FOR RESISTANCE TO CERCOSPORA LEAF SPOTS AND RUST DISEASES IN KANO STATE, NIGERIA

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Abstract

***Cercospora* leaf spots and rust diseases are among the biotic factors that cause yield reduction in groundnut. However, screening groundnut breeding lines that are tolerant to these diseases and having higher yield potentials would help farmers and breeders to make the right choice for cultivation or for further improvement. Therefore, this study was conducted to evaluate 18 groundnuts advanced breeding lines and 2 improved/local varieties against these diseases, during July-October 2016 rainy season in a field designated as endemic to *Cercospora* leaf spots and rust diseases, at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Research Farm at Minjibir, Kano, Nigeria. Significant differences ($p<0.01$) were observed in all the traits studied (*viz*., days to 50% flowering, normalised difference vegetation index, pod yield (Kg/ha), fodder yield (Kg/ha), 100 seed weight (g), disease incidence (%) and disease severity) except chlorophyll content. The top nine (9) high yielding breeding lines (ICGV's-IS 13980, 07947, 07828, 09011, ICGX-IS's 11003, 11057, 13011, ICGV-SM 07539 and ICG 5891) were all resistant to early leaf spot, moderately resistant to late leaf spot and rust except ICGV's-IS 07828, 09011 and ICGX-IS 13011 which were susceptible to rust.**

Key words: *Cercospora,* Rust, Groundnut, Resistance, Yield.

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Introduction

Groundnut (*Arachis hypogaea* L.) also known as Peanut (Beghin et al., 2003) is a member of the genus *Arachis* in the family *Fabacaea. Arachis* species are native to South America, originated from central Brazil, which is now cultivated in more than 100 countries, covering an area of 26.4 million hectare (Beghin et al., 2003).

Arachis hypogaea is an economically valuable oilseed and cash crop grown extensively in the savannah region of Nigeria, majorly for direct consumption as food and industrial use (Abalu, 1996). The fodder is used as feed for livestock, especially during dry seasons (Tsigbey et al., 2013). According to International Nut and Dried fruit (INC), the world annual production in 2014 was more than 35.6 million metric tons (INC, 2014-15). The leading producers are China, India, Nigeria, U.S.A, Senegal, Indonesia and Sudan (Food and Agricultural Organisation [FAO], 2004). Yields of groundnut have been reported as low in developing countries compared to developed countries due to climatic, cultural and biotic factors including diseases (Janila et al., 2013).

Among the diseases, leaf spots and rust has the greatest impact on groundnut yield which may cause more than 50% yield losses (Waliyar, 1991). Early leaf spot caused by *Cercospora arachidicola* Hori (*Mycosphaerella arachidis* Deighton), late leaf Spot caused by

Phaeoisariopsis personata (Berk and Curtis) Van Arx and rust caused by *Puccinia arachidis* (Speg) are economically significant worldwide (Grichar et al., 1998). Significant pod yield losses have been reported due to effect of each of these diseases especially under favourable conditions. For example, early leaf spot alone can cause 35-50% leaf defoliation at the peak of flowering stage and yield losses may reach up to 20-25% (Mehan & Hong, 2001). Up to 80% of the pod yield losses are reported due to late leaf spot (Grichar et al., 1998). Early leaf spot is characterised by the presence of light brown to black lesions with chlorotic yellow halo on the upper surface of the leaves (Nutter, 1995). The late leaf spot is characterised by dark brown to black circular lesion (usually without halo) on the lower surface of the leaves and can be formed on the stems and petioles (Damicone, 1990). The rust pathogen produces orange uredinial pustules on the lower leaf surfaces that rupture to expose the reddish brown urediniospores. The combined effect of *Cercospora* (early and late) leaf spots and rust could result in disease severity as high as 80% defoliation at harvest (Waliyar, 1991). These losses could be greatly reduced by adopting variety of methods in disease management, in which host plant resistance could be a cheap and reliable and method. Jordan et al. (2013) indicated that host plant resistance has been preferred to other methods in leaf spots and rust management due to its cost effectiveness and environmental friendliness. However, report indicates that disease resistance in groundnut is mostly associated with low yield, poor pod formation, poor kernel characteristics and late maturity which make breeding for leaf spot resistance difficult (Singh et al., 1997; Subrahmanyam et al., 1995). Varieties that are resistant to *Cercospora* leaf spots and rust are very useful to farmers in order to get high yield and maximise profit, and could also serve as good parental sources for groundnut improvement programmes. There is therefore the need for routine screening of some groundnut breeding lines for resistance to these diseases. Hence, this study is designed to evaluate some groundnut advanced breeding lines by screening against foliar fungal diseases under natural infection.

Materials and Methods

Experimental Site and Design

The experiment was conducted during the 2016 rainy season at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Research Farm at Minjibir, Kano (latitude 12º10¹42N, longitude 8º39¹33E). The treatment comprised of 18 advanced groundnut breeding lines and 2 improved/local varieties (Samnut 25 and Kwankwaso) as checks. These were laid out in a Randomised Complete Block Design (RCBD) with 3 replicates. The experimental field was ploughed, harrowed and ridged to 75 cm (0.75 m) apart, then marked out into four (4) blocks per replicate, each block was divided into 5 plots with a size of 6.0 m^2 (4x1.5 m). Groundnut seeds were sown at 75 cm inter row and 10 cm intra row spacing with 1 seed per hole (making a total of 80 stands per plot). Precaution was taken to ensure uniform and proper depth of planting (5 cm).

Data Collected

Days to 50 per cent flower

This was carried out by counting the number of days from sowing to when 50 per cent of the stands in a plot flowered.

Chlorophyll content

Three plants per plot were selected randomly at 62 days after sowing DAS (flowering), a leaf from the 2^{nd} to 4^{th} uppermost fully expanded leaves was chosen, and one of its leaflets was selected and read using chlorophyll meter (SPAD-502 plus) once.

Normalized difference vegetation index (NDVI)

The NDVI was evaluated using GreenSeeker device, by passing it over the top of the plants at a height of approximately 0.50 m, of the two rows of each plot. This was carried out when the crops were fully developed, at 100 DAS (pod maturation).

Disease incidence

Incidence of early leaf spot was assessed at 40 days after sowing (DAS), late leaf spot at 65 DAS and rust at 75 DAS by counting the number of plants infected and expressing it as a percentage of the total number of plants per plot as given as

$$\text{Disease incidence (\%)} = \frac{\text{No. of diseased plants}}{\text{Total no. of plants examined}} \times \frac{100}{1}$$

Disease Severity

Disease severity (early and late leaf spots and rust) was assessed based on a rating scale of increasing severity of 1-9 (Subrahmanyam et al., 1995). Disease score 1 means 0% foliar infection; 2 (1-5%); 3 (6-10%); 4 (11-20%), 5 (21-30%); 6 (31-40%); 7(41-60%), 8 (61-80%) and 9 (81-100%) of foliar area infection with plants having almost all leaves defoliated leaving bare stems. Breeding lines with a disease score 4-5 were considered moderately resistant and 6 were designated as susceptible as reported by

Sudini et al., 2015.

Yield/Yield Components
Dry Weight of Pods per Hectare

After hand picking the pods, they were then sun dried on the ground to less than 10% moisture content, the dried and cleaned (removal of pegs, leaf debris, sand, diseased and unfilled pods etc.) pods were weighed to Kg per plot and extrapolated to Kg per hectare.

Dry Weight of Fodder per Hectare

After hand picking the pods, the haulms were dried in the field for 4-5 days. The dried haulms were weighed per plot and expressed in Kg per hectare.

100 Seed Weight

This was carried out by counting 100 seed per plot and weighed using electronic weighing balance.

Statistical analysis

The data collected were summarised using Microsoft Excel software. Percentage data were transformed using arcsine transformation in Microsoft Excel prior to analysis of variance. GenStat Statistical Software (17.0 edition) was used for analysis of variance and to find correlations among means. Means were separated at 5% level of probability using Student Newman Keuls (SNK).

Results

Agronomic and Yield/Yield Components

Significant differences ($p<0.01$) were observed for the agronomic parameters recorded on the 18 groundnut advanced breeding lines and 2 improved/local varieties. The mean number of days to 50% flowering was 28 DAS. The breeding lines and a check ICGV-IS 07965, ICGV-SM 07541, and Kwankwaso had the longest days to 50% flowering while ICGX-IS 11003 had the lowest. The breeding line ICGV-IS 13863 recorded the highest NDVI whiles ICGV-IS 07919 and ICGX-IS 13011 had the lowest. There was no significant difference on chlorophyll content (SPAD). However, ICGX-IS 13011 recorded the highest chlorophyll content while, Samnut 25 had the lowest (Table 1). Significant differences were observed for the yield/yield component parameters (figure 1), ICGV-IS 09011 had the highest pod yield while, ICGV-SM 07541 has the highest fodder yield (Kg/ha). However, ICGV-SM 08568 recorded the lowest pod yield. In addition, ICGV-IS 13980 had the heaviest 100 seed weight whiles ICGV-SM 08568 had the lowest.

Table 1: Number of Days to 50% Flowering, Chlorophyll Content and Normalised Difference Vegetation Index of 18 Advanced Breeding Lines and 2 Improved/Local varieties

Advanced Breeding lines	Days to 50% flowering	Chlorophyll content	Normalise diff. veg. Index
ICG 12991	27.33abc	37.27	0.73a-d
ICG 5891	28.67bcd	37.13	0.80ab
ICGV-IS 07803	26.67abc	34.3	0.80ab
ICGV-IS 07828	27.00abc	30.5	0.76a-d
ICGV-IS 07850	30.00cd	30.1	0.71a-d
ICGV-IS 07919	29.67cd	34.27	0.61d
ICGV-IS 07947	28.67bcd	35.7	0.75a-d
ICGV-IS 07965	31.33d	30.4	0.75a-d
ICGV-IS 09011	28.67bcd	33.4	0.83ab
ICGV-IS 13863	26.00ab	36.67	0.86a
ICGV-IS 13980	30.00cd	35.23	0.79abc
ICGV-SM 07539	30.00cd	35.8	0.66bcd
ICGV-SM 07541	31.33d	37.67	0.71a-d
ICGV-SM 08568	29.33bcd	35.63	0.69a-d
ICGX-IS 11003	25.00a	32.5	0.78a-d
ICGX-IS 11057	27.33abc	30.17	0.75a-d
ICGX-IS 13011	30.00cd	37.97	0.62cd
ICGX-IS 13988	27.33abc	33.83	0.76a-d
KWANKWASO	31.33d	34.4	0.75a-d
SAMNUT 25	26.00ab	29.27	0.76a-d
Means	28.58	34.11	0.74
CV%	4.3	12.6	7.8
SE	0.712	2.473	0.034

Means followed by the same letter(s) in a column are not significantly different at 5% level of probability using Student-Newman Keuls Test (SNK).

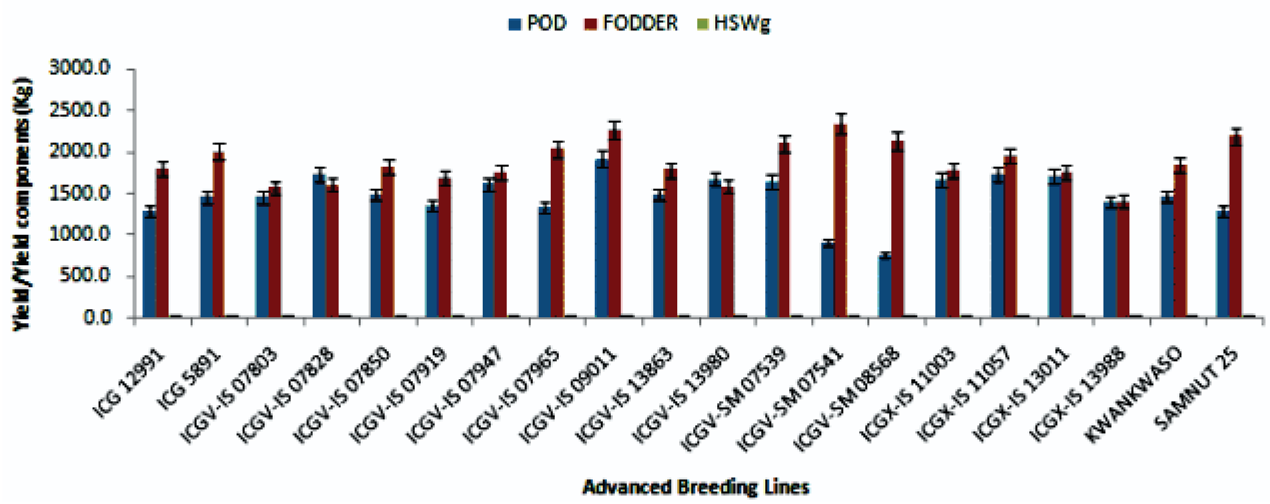

Figure 1: Yield/Yield Components of 18 Groundnut Advanced Breeding Lines and 2 Improved/Local varieties. HSW= Hundred Seed Weight.

Disease Components

Disease incidence among 18 advanced breeding lines and 2 improved/local varieties of groundnut

Significant difference ($p<0.01$) was observed among the groundnut advanced breeding lines and improved/local varieties with respect to incidence of early, late leaf spot and rust diseases (figure 2). At 40, 65 and 75 DAS, ICGV-IS 07919 had the lowest incidence while, ICGV-IS 09011 and ICGV-SM 07539 had the highest. Significant contribution and associations of one disease to another was also observed among breeding lines. By 75 DAS all plants (100%) had been infected with early, late leaf spot and rust diseases respectively but at different degrees of severity.

Disease severity among 18 advanced breeding lines 2 improved/local varieties of groundnut

Significant differences ($p<0.05$) were observed among the groundnut advanced lines and improved/local varieties with respect to disease severities (Table 2). The mean severity score was 2.73, 4.35 for early/late leaf spot and 5.84 for rust at 40, 65 and 75 DAS respectively. ICGV-IS 07965 and ICGV-SM 08568 had the lowest severity score for early and late leaf spots whiles, ICGV-IS 13863 had the highest for early leaf spot and ICGV-IS 07828 for late leaf spot. However, Kwankwaso had the lowest severity score for rust while, ICGV-IS 07803 had the highest.

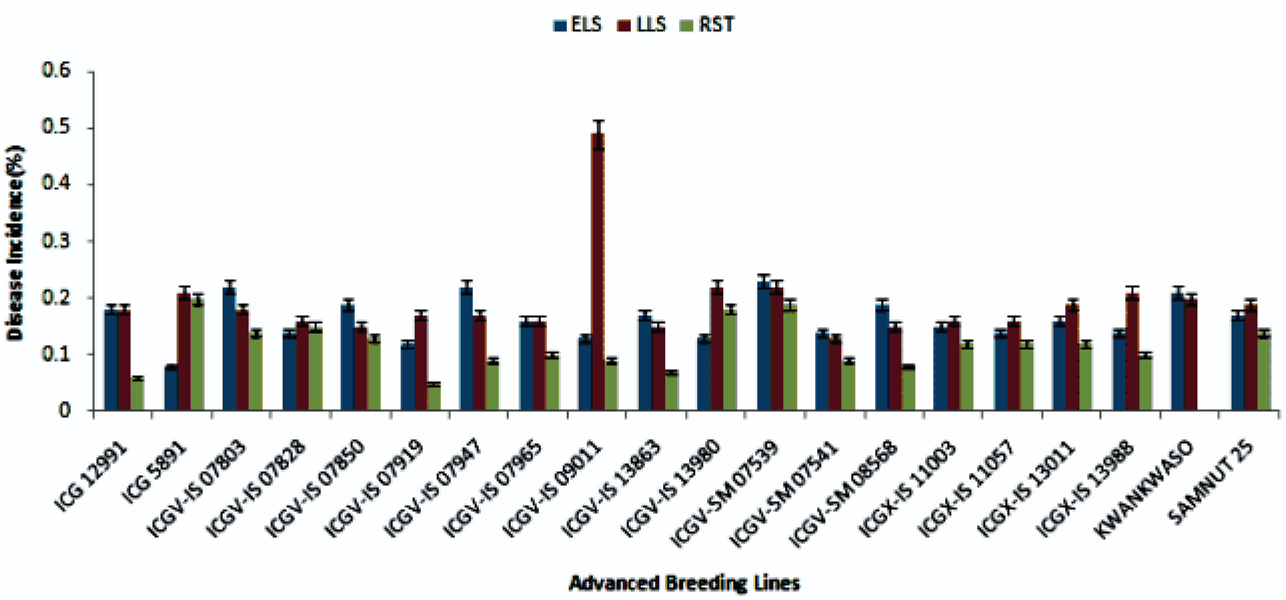

Figure 2: Cercospora Leaf Spots and Rust Disease Incidence on 18 Advanced Breeding Lines and 2 Improved/Local varieties. ELS= Early Leaf Spot, LLS= Late Leaf Spot, RST= Rust.

Table 2: Disease Severity Score on 18 Advanced Breeding Lines and 2 Improved/Local varieties

Advanced Breeding Lines	Early Leaf Spot	Late Leaf Spot	Rust
ICG 12991	3.0	4.5	6.7
ICG 5891	2.6	4.3	5.0
ICGV-IS 07803	3.0	4.9	8.3
ICGV-IS 07828	2.5	4.8	6.7
ICGV-IS 07850	2.5	4.4	7.0
ICGV-IS 07919	2.8	4.4	6.0
ICGV-IS 07947	2.7	4.0	5.3
ICGV-IS 07965	2.2	3.7	6.0
ICGV-IS 09011	3.1	4.4	6.7
ICGV-IS 13863	3.8	4.7	3.0
ICGV-IS 13980	2.7	4.4	6.0
ICGV-SM 07539	2.2	4.4	6.0
ICGV-SM 07541	2.4	4.3	5.0
ICGV-SM 08568	2.4	3.9	6.0
ICGX-IS 11003	2.7	4.3	7.0
ICGX-IS 11057	2.5	4.1	6.3
ICGX-IS 13011	2.9	4.8	6.3
ICGX-IS 13988	3.1	4.4	5.3
KWANKWASO	2.7	4.2	1.0
SAMNUT 25	2.7	4.0	7.0
Means	2.73	4.35	5.84
CV%	23.8	9.2	31.0
SE	0.375	0.231	1.045

Breeding lines with disease score of 6 and above were designated as susceptible to a particular disease.

Table 3: Performance of Groundnut Advanced Breeding Lines and Improved/local varieties against Cercospora (Early and Late) Leaf Spotsand Rust Diseases

Reaction	Disease	Number of Breeding Lines and Details
Resistant	Early leaf spot	20 Advanced Breeding Lines/Checks: ICG 12991, ICG 5891, ICGV-IS 07803,ICGV-IS 07828, ICGV-IS 07850, ICGV-IS 13863, ICGV-IS 07919, ICGV-IS 07947, ICGV-IS 07965, ICGV-IS 09011, ICGV-IS 13980, ICGV-SM 07539, ICGV-SM 07541, ICGV-SM 08568, ICGX-IS 11003, ICGX-IS 11057, ICGX-IS 13011, ICGX-IS 13988, Kwankwaso, Samnut 25
	Late leaf spot	2 Advanced Breeding Lines: ICGV-IS 07965, ICGV-SM 08568
	Rust	2 Advanced Breeding Lines/Check: ICGV-IS 13863, Kwankwaso
	Early/Late leaf spot and Rust	Nil
Moderately Resistant	Early leaf spot	Nil
	Late leaf spot	18 Advanced Breeding Lines/Checks: ICG 12991, ICG 5891, ICGV-IS 07803,ICGV-IS 07828, ICGV-IS 07850, ICGV-IS 07919, ICGV-IS 09011, ICGV-IS 07947, ICGV-IS 13980, ICGV-SM 07539, ICGV-SM 07541, ICGX-IS 11003, ICGX-IS 11057, ICGX-IS 13011, ICGX-IS 13988, Kwankwaso, Samnut 25, ICGV-IS 13863
	Rust	9 Advanced Breeding Lines: ICG 5891, ICGV-IS 07947, ICGV-SM 07541, ICGX-IS 13988, ICGV-IS 07965, ICGV-IS 13980, ICGV-SM 07539, ICGX-IS 11003, ICGX-IS 11057.
	Early/Late leaf spot and Rust	Nil
Susceptible	Early leaf spot	Nil
	Late leaf spot	Nil
	Rust	8 Advanced Breeding Lines/Check: ICG 12991, ICGV-IS 07828, ICGV-IS 07850, ICGV-IS 07919, ICGV-IS 09011, ICGV-SM 08568, ICGX-IS 13011, Samnut 25
	Early/Late leaf spot and Rust	Nil
Highly-Susceptible	Early leaf spot	Nil
	Late leaf spot	Nil
	Rust	1 Advanced Breeding Line: ICGV-IS 07803
	Early/Late leaf spot and Rust	Nil

Breeding lines are categorised based on mean disease severity of resistance/susceptible reaction to early and late leaf spot and rust disease on a 1-9 rating scale where 1-3=Resistant, 4-5=Moderately resistant, 6-7=Susceptible, 8-9=highly susceptible.

Association between agronomic traits and disease severity

The agronomic/yield parameters of the groundnut advanced breeding lines viz. days to 50% flowering, normalised difference vegetation index, pod yield (Kg/ha), fodder yield (Kg/ha) and 100 seed weight (g) were positively associated to each other except chlorophyll content (SPAD), but negatively associated with leaf spots and rust disease severities. Disease components significantly associated among themselves (Table 4).

Table 4: Matrix of Simple Correlation of Foliar Diseases Severities and Yield/Yield Components of Groundnut Advanced Breeding Lines and Improved/Local Varieties

Varieties	POD (Kg)	FODDER (Kg)	HSW (g)	SPAD	NDVI	ELS-SV	LLS-SV	RST-SV
POD(Kg)	1.000							
FODDER(Kg)	0.073	1.000						
HSW(g)	0.340**	0.029	1.000					
SPAD	0.040	-0.017	-0.030	1.000				
NDVI	0.127	0.009	0.058	0.112	1.000			
ELS-SV	-0.068	-0.398 **	-0.009	0.114	0.113	1.000		
LLS-SV	-0.241*	-0.343 **	0.197	0.080	0.132	0.302 **	1.000	
RST-SV	-0.081	-0.028	-0.311 **	-0.042	-0.020	0.084	0.271*	1.000

*, **Significant at p≤ 0.05 and p≤ 0.01 respectively, POD=pod yield, FODDER= fodder yield, HSW=hundred seed weight, SPAD=chlorophyll content, NDVI=normalised difference vegetation index, ELS-SV=early leaf spot severity, LLS-SV=late leaf spot severity, RST-SV=rust severity.

Discussion

The significant differences observed in this study, indicate that agronomic variation exists across the 18 advanced breeding lines and 2 improved/local varieties of groundnut. This could be due to differences in their genetic composition. Similarly, Upadhyaya (2003) asserted that agronomic traits are useful in describing how a particular groundnut genotype is different from the other (for example ICGX-IS 11003 reached 50% flowering earlier than either of the checks, also ICG 5891 had highest chlorophyll content and NDVI than Kwankwaso or Samnut 25). In a previous study, Gaikpa et al. (2015) found molecular variation across genotypes of groundnuts. Janila et al. (2013) maintained that pod yield per plant and 100 seed weight are very important yield contributing parameters. This explains the significant and high positive correlation between the agronomic traits, for example, hundred seed weight is significant and positively correlated with pod yield, also there is positive correlation between fodder and pod yield though is not significant in this study. The advanced breeding lines and improved/local varieties showed significant and different levels of resistance to *Cercospora* leaf spots and rust diseases. This may be due to their genetic resistance or tolerance to the disease or their ability to produce some level of phenolic compound which helps in inhibition of spore germination (Wang et al., 2012). The negative associations of disease parameters to yield and yield components show that the disease affected the yield potential of the groundnut (for example, ELS, LLS, RST were significant and negatively correlated to both pod and fodder yield). All high yielding advanced lines in this study were resistant to moderately resistant. Despite the level of resistance or tolerance observed across the advanced breeding lines and improved/local checks, it is also important to note that the disease had effect on the overall pod yield since none of the advanced breeding lines or improved/local checks had a pod yield up to the 3000 Kg/ha. Yield reduction in groundnut as a result of *Cercospora* leaf spots disease has been reported in most cases (Tsigbey et al., 2013; Waliyar, 1991). This is because the *Cercospora* pathogens have been found to produce cercosporin during their early growth stages which causes lipid peroxidation and breakdown of photosynthetic pigments of plant tissues leading to inefficient dry matter production and partitioning (Shabana et al., 2013). The pathogens have also been found to destroy the stomata pore with their germ tubes (Shokes and Culbreath, 1997). Significant yield variations were found across the 18 groundnut advanced breeding lines and 2 improved/local varieties grown under the *Cercospora* leaf spots and rust natural disease pressure and none of the improved or local variety (checks) gave higher yield above either of breeding lines. The high yielding breeding lines were all resistant to early leaf spot, moderately resistant to late leaf spot and rust except ICGV's-IS 07828, 09011 and ICGX-IS 13011 which were susceptible to rust.

This finding will be very useful to breeders in the selection of breeding lines with good agronomic traits in combination with disease resistance to the *Cercospora* leaf spots and rust in their breeding programmes. In addition, these breeding lines could be cultivated or disseminated to areas with high levels of *Cercospora* leaf spots and rust disease pathogens.

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