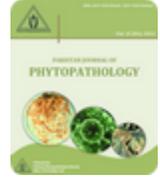




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## EVALUATION OF GROUNDNUT (*ARACHIS HYPOGAEA* L.) MUTANT GENOTYPES FOR RESISTANCE AGAINST MAJOR DISEASES OF GROUNDNUT

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### ABSTRACT

A field experiment was conducted at the Farming for the Future field of the Faculty of Agriculture, Nyankpala campus of the University for Development Studies, UDS, to evaluate groundnut genotypes for resistance against three major groundnut diseases namely early leaf spot (ELS) *Cercospora arachidicola*, late leaf spot (LLS) *Phaeoisariopsis personata* and groundnut rosette disease under natural infestation. The experiment was consisted of 6 treatments including four groundnut genotypes (150 Gy, 200 Gy, 250 Gy, and 300 Gy) and two standard checks (Chinese and Nkatie SARI varieties). The treatments were replicated three times in a randomized complete block design. Data were collected on ELS and LLS severity using a modified nine point scale (1-9), while rosette disease score was assessed using a 1-5 disease rating scale. Yield of groundnut and resistance level were also determined at 85 DAP. The results revealed that, mutant genotypes 250 Gy and 200 Gy had the highest yield compared with the other genotypes. The 250 Gy was found to be resistant against all the three major groundnut diseases studied, while the 150 Gy, 200 Gy, 300 Gy and the two controls exhibited moderate resistance. Genotype 200 Gy demonstrated resistance against the rosette disease, while the 150 Gy and 300 Gy showed moderate resistance and the two controls were susceptible to groundnut rosette disease. The ELS and LLS diseases correlated negatively with pods per plant and grain yield, respectively, while the rosette disease showed negative correlation with both pods per plant and grain yield. Genotype 250 Gy was resistant against the three major groundnut diseases, and can be further used in future analytical breeding studies to identify and assess specific genes responsible for the resistance, at molecular level for advanced improvement.

**Keywords:** Mutagenesis, early leaf spot, late leaf spot, groundnut rosette disease, incidence.

### INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a leguminous crop which is now grown in both the tropical and subtropical countries as oil seed (Ephrem, 2015). It is cultivated on subsistence and commercial bases for food and industrial purposes (Thakur *et al.*, 2013; Mohammed *et al.*, 2018). The general productivity of groundnut in Africa is comparatively low, compare to that of America due to a number of constraints such as biological,

undulating climate, socio-economic factors, pests and diseases (Zongo *et al.*, 2019). The average output of groundnut in Africa (902.6 kg/ha) is very low comparative to the American output of 33381.4 kg/ha (FAOSTAT, 2018). The average yield of groundnut in Africa is about ¼ of that of the America's and about ½ of the yield of Asia due to low mechanization, pressure of pest and diseases and low yielding varieties among others (Desmae and Sones, 2017). Groundnut is considered as the most valuable crop among legumes, grown in northern Ghana because of its economic, dietary and industrial advantages (Sugri, 2020).

Mohammed *et al.* (2019) reported that, the combination of leaf spot, rosette disease and drought can lead to yield loss of 100% in groundnut. Number of groundnut

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germplasm has been reported having various degree of resistance to the early leaf spot, late leaf spot and rosette disease of groundnut, however, knowledge about resistance level exhibited by the germplasm is not sufficient (Mohammed *et al.*, 2018). The early leaf spot has symptoms of brown to reddish brown colour usually surrounded by yellow halo on the upper surface of the leaf while late leaf spot has symptoms of dark brown to black spots, normally with no yellow halo on the lower surface of the leaf (Desmae and Sones, 2017). Total resistance to the early and late leaf spots fungi has not yet been identified (Zongo *et al.*, 2019).

The groundnut rosette virus disease is native to Sub-Saharan Africa and its endemic has been observed in Burkina Faso, Ghana, Nigeria, Malawi, Mozambique, Mali, Niger Republic, Uganda, Kenya and Tanzania (Thuo *et al.*, 2014). Kayondo *et al.* (2014) reported rosette associated annual yield loss of US\$ 156 million in Sub-Saharan Africa, according to the estimation of the International Crop Research Institute for Semi-Arid Tropics. It occurs in an alarming rate annually and negatively affects rural economic growth in Sub-Saharan Africa (Mukoye and Mabele, 2019). The groundnut rosette disease is caused by three individual viral components: groundnut rosette virus (GRV), satellite RNA (SAT RNA) and groundnut rosette assistor virus (GRAV) (Kayondo *et al.*, 2014) and transmitted by the *Aphis craccivora* Koch. It has two types of symptoms, the chlorotic rosette that has been observed in Sub-Saharan Africa since introduction of groundnut into this region and the green rosette form has only been present in West African nations (Mugisa *et al.*, 2016). A third symptom of mosaic rosette has also been observed in the areas of East and Southern Africa (Mabele *et al.*, 2018). The chlorotic rosette have bright yellow colored leaves with a small spot of green and the green rosette have light green to dark green mosaic with leaf margins curled downwards (Desmae and Sones, 2017).

The most important problem encountered in the groundnut cultivation areas in Northern Ghana is the incidence of diseases, notably the early and late leaf spots which are caused by *Cercospora arachidicola* and *Phaeoisariopsis personata* (Frimpong *et al.*, 2006b). These two leaf spots diseases are key biological factors against groundnut production in Northern Ghana (Alidu *et al.*, 2019). The most common and commercial variety, (Chinese) grown in the Guinea savannah of Ghana is highly vulnerable to these diseases (Alidu *et al.*, 2019).

The application of induced mutation has led to the generation of new and beneficial modifications of characteristics features of plants including high yielding and disease resistance qualities. Gamma rays, a physical mutagen from the ionizing radiation group, used in mutation breeding can have direct actions on the molecular composition of the cell (Gaswanto *et al.*, 2016). Gamma rays also act on free moving radicals transported by water, which can negatively impact or transform fundamental cellular constituents of plant and several studies have shown that differential influences from gamma radiations can alter structural morphology and physio-chemical characteristics of plants (Kim *et al.*, 2004).

Lack of resistant variety to these three major diseases encouraged the continuous use of chemicals to control it (Khan *et al.*, 2013). In groundnut production area like northern Ghana, groundnut is extensively cultivated by peasant farmers who are financially weak to afford the chemical control method during the growing season (Adu-Dapaah *et al.*, 2004). The use of chemicals to control plant pathogens causes water and soil pollutions as well as destruction of beneficial soil microorganisms (Rani *et al.*, 2017). Though chemical control has proven to be effective for increasing yield, it can potentially cause harm to human and environment through residues (Al-Ahmadi, 2019). The severity of these diseases sometimes calls for some farmers in the rural settings of northern Ghana to use some un-approved and highly hazardous agrochemicals of which they have only little or no knowledge about and also lack entirely the requisite training in relation to mode of application and safety measures. Hence, one of the most appropriate alternatives to combat diseases of crop plants is the use of biologically engineered cultivars having resistance (Rani *et al.*, 2019). According to Desmae and Sones (2017) the best approach to overcome leaf spots disease of groundnut is the use of resistance varieties.

A number of resistant lines of groundnut have been found in several studies (Monyo and Varshney, 2016). Muduli and Misra (2007) also reported that, the exposure of genetic material such as seed, callus and pollen to irradiations facilitate the likelihood for obtaining peculiar genetic material, which can generate the variations of quantitative and qualitative crops heritable features. This research was particularly aimed at determining groundnut mutant genotypes resistant to major groundnut diseases (the early leaf spot, late leaf

spot and the rosette disease) under natural field infestation in order to minimize yield losses caused by these diseases.

**MATERIALS AND METHODS**

**Experiment Site:** Field experiment was carried out during the 2018/2019 cropping season, from June to October at the University for Development Studies, Faculty of Agriculture's Faming for the Future field at Nyankpala in the Tolon district of the northern region of Ghana, about 20 kilometers South-West of Tamale. Nyankpala is located between 9° 24' 0" North and 0° 59' 0" West and found in the guinea savannah agro-ecological zone of Ghana. The area experiences a monomodal rainfall, with an annual rainfall of about 1000-1200 mm dispensed evenly from April to November. It has a uniform temperature distribution with the average minimum and maximum of 21C° and 32C° respectively per month (SARI Annual Report, 2012). The soil is sandy-loam, moderately drained, contains no hard mass and formed from Voltarian rock which is described as Nyankpala series (SARI Annual Report, 2012).

**Sources of groundnut genotypes:** The groundnut

genotypes (150 Gy, 200 Gy, 250 Gy and 300 Gy) and the Chinese variety (1st control) were obtained from the Department of Agronomy, Faculty of Agriculture at the Nyankpala campus of the University for Development Studies (UDS). The Nkatie SARI variety (2nd control) was obtained from the Council for Scientific and Industrial Research Institute-Savannah Agriculture Research Institute (CSIR-SARI), Nyankpala in the Northern region of Ghana.

**Experimental design:** The experiment was laid out on the field in a Randomized Complete Block Design (RCBD), with six treatments; four groundnut genotypes and two controls replicated three times. The plot size was 3 m × 3 m = 9 m<sup>2</sup>. The alleys between plots and replications were 1 m and 1.5 m respectively. In all, there were 18 experimental units and the total area for the study was; 12 m × 23 m = 276 m<sup>2</sup> = 0.0276 ha.

**DATA COLLECTION**

**Disease incidence:** Early leaf spot (ELS), late leaf spot (LLS) and rosette diseases affected plants in each plot were counted and expressed as a percentage of the total groundnut plants on the plot. It was calculated by the formula below (Waliyar *et al.* (2007).

$$PDI = \frac{\text{Number of plants showing disease symptom}}{\text{Total number of plants per plot}} \times 100$$

**Early and late leaf spots severity:** Early leaf spot and Late leaf spot disease severity scorings were done at 45 and 85 days after planting (DAP) using a modified 9-point scale (Subrahmanyam *et al.*, 1995) (Table 1),

where a score of 1 was rated as highly resistant (HR), 2 to 4 as resistant (R), 5 and 6 as moderately resistant (MR), 7 and 8 as susceptible and 9 as highly susceptible (HS).

Table 1 Modified 9-point scale used for field screening of groundnut genotypes for resistance to ELS and LLS

Disease score	Description of disease	Severity (%)
1	No disease	0
2	Lesions present largely on lower leaves; no defoliation.	1-5
3	Lesions present largely on lower leaves, very few on middle leaves; defoliation of some leaflets evident on lower leaves.	6-10
4	Lesions on lower and middle leaves but severe on lower leaves; defoliation of some Leaflets evident on lower leaves.	11-20
5	Lesions present on all lower and middle Leaves; over 50% defoliation of lower leaves.	21-30
6	Severe lesions on lower and middle leaves; lesions present but less severe on top leaves; extensive defoliation of lower leaves; defoliation of some leaflet evident on middle Leaves.	31-40
7	Lesions on all leaves but less severe on top leaves; defoliation of all lower and some middle leaves	41-60
8	Defoliation of all lower and middle leaves; severe lesions on top leaves; some defoliation of top leaves evident.	61-80
9	Almost all leaves defoliated, leaving bare Stems, some leaflets may remain, but show severe leaf spots.	80+

(Subrahmanyam *et al.*, 1995; Wilber, 2014)

**Rosette disease severity:** Groundnut rosette disease (GRD) severity was assessed by scoring at 85 days after planting (DAP) using a 1-5 disease rating scale (Pande *et al.*, 1997;

Olorunju *et al.*, 2001) (Table 2). Where 0 = Highly resistant (HR), 1 = Resistant (R), 2 = Moderately resistant (MR), 3 = Susceptible (S) and 4 = Highly susceptible (HS).

Table 2: A 1-5 disease rating scale for scoring the severity of the rosette disease of groundnut

Scale	Description of disease
0	No visible symptoms on leaves
1	Rosette symptoms on 1-20% leaves, but no obvious stunting
2	Rosette symptoms on 21-50% leaves with stunting
3	Severe symptoms on 51-70% leaves with stunting
4	Severe symptoms on 71 to 100% leaves with stunting

(Pande *et al.*, 1997; Olorunju *et al.*, 2001)

**Number of pods per plant:** A sample of five plants was selected at random from each plot at harvest and their

pods were plucked together, counted and the average was taken.

$$\text{Formula} = \frac{\text{Sum of pods from selected plants}}{\text{Number of selected plants}}$$

**Total grain yield:** Pods were plucked from all the groundnut plants per each plot. The pods were sun-dried for four days and weighed in kilograms. The weight in kilogram per plot was further extrapolated to kilogram per hectare scale, to obtain the average grain yield per hectare.

spots diseases were significantly different ( $P < 0.05$ ). The 250 Gy recorded the least percentages of early and late leaf spots incidences (Figure 1). Rosette disease incidence was also significantly different ( $P < 0.05$ ). The 200 Gy and 250 Gy recorded the least percentages and the controls (Chinese and the Nkatie SARI) gave the highest percentages. There were no significant differences in the percentages of ELS, LLS and rosette incidences among the other genotypes. However, the percentages of rosette incidence recorded by these genotypes were significantly different from that of the 200 Gy and 250 Gy (Figure 1).

**DATA ANALYSIS**

All data collected were subjected to analysis of variance (ANOVA). Genstat (18<sup>th</sup> edition) statistical package was used in the analysis. Means were separated using the Least Significant Difference test (LSD) at 5% probability level.

**RESULTS**

**Disease incidence:** The incidences of early and late leaf

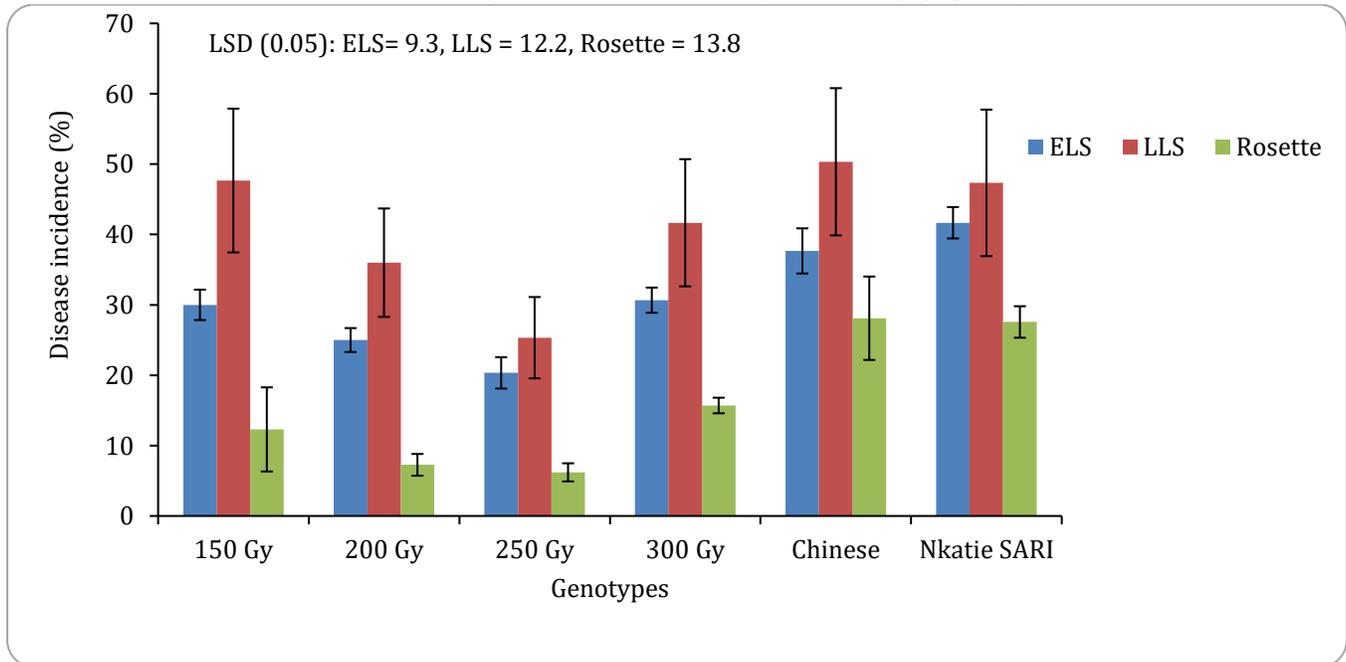


Figure 1. Reactions of groundnut mutant genotypes to major diseases of the crop. Error bars represent standard error of means (SEM).

**Severity of major groundnut diseases:** The severity of the ELS disease among the mutant genotypes was not significantly different ( $P > 0.05$ ). However, the 250 Gy recorded the least mean severity score, which was significantly different from the other genotypes and the two controls (Chinese and Nkatie SARI). The severity of the LLS showed a significant difference ( $P < 0.05$ ) among the groundnut genotypes. Genotype 250 Gy was resistant against the ELS and LLS diseases, whilst the other genotypes and the controls were moderately resistant (Table 3).

There were significant differences ( $P < 0.05$ ) in severity score for the rosette disease among the genotypes (Table 3). Mutant genotypes 250 Gy had the least rosette severity score. Mutant genotype 250 Gy was also significantly different ( $P < 0.05$ ) compared with the genotypes 150 Gy, 300 Gy and the two controls (Chinese and Nkatie SARI), except genotype 200 Gy, in the mean disease severity score and the corresponding resistance level. Mutant genotypes 150 Gy, and 300 Gy were moderately resistant against rosette disease, whilst the Chinese and the Nkatie SARI were susceptible to groundnut rosette disease (Table 3).

Table 3: Disease severity for ELS, LLS and GRD for groundnut genotypes

Treatment	ELS	LLS	GRD
150 Gy	4.6 (MR)	6.1 (MR)	2.2 (MR)
200 Gy	4.9 (MR)	6.0 (MR)	1.4 (R)
250 Gy	3.7 (R)	4.2 (R)	1.0 (R)
300 Gy	4.5 (MR)	5.9 (MR)	2.0 (MR)
Chinese	5.1 (MR)	6.1 (MR)	3.1 (S)
Nkatie SARI	5.2 (MR)	5.9 (MR)	2.6 (S)
LSD (0.05)	1.2	1.3	1.0
CV (%)	14.2	12.6	26.2

Where: ELS Means Early leaf spot, LLS Means Late leaf spot and GRD Means Groundnut rosette disease. Resistance level with respect to the peak of diseases severities, where: R = Resistance, MR = Moderate Resistance, S = Susceptible, as defined in Table 1 and 2 for early and late leaf spot and rosette respectively.

**Number of pods per plant:** The incidence of diseases studied had a significant influence ( $P < 0.05$ ) on the average number of pods per plant. Mutant genotype 250 Gy recorded more pods per plant while the 300 Gy recorded least number of pods per plants (Figure 2). The

pod per plant of the 250 Gy was significantly different from that of the 300 Gy, 150 Gy and the Chinese (1st control). However, the pod per plant of the 200 Gy and Nkatie SARI (2nd control) were not different from the 250 Gy (Figure 2).

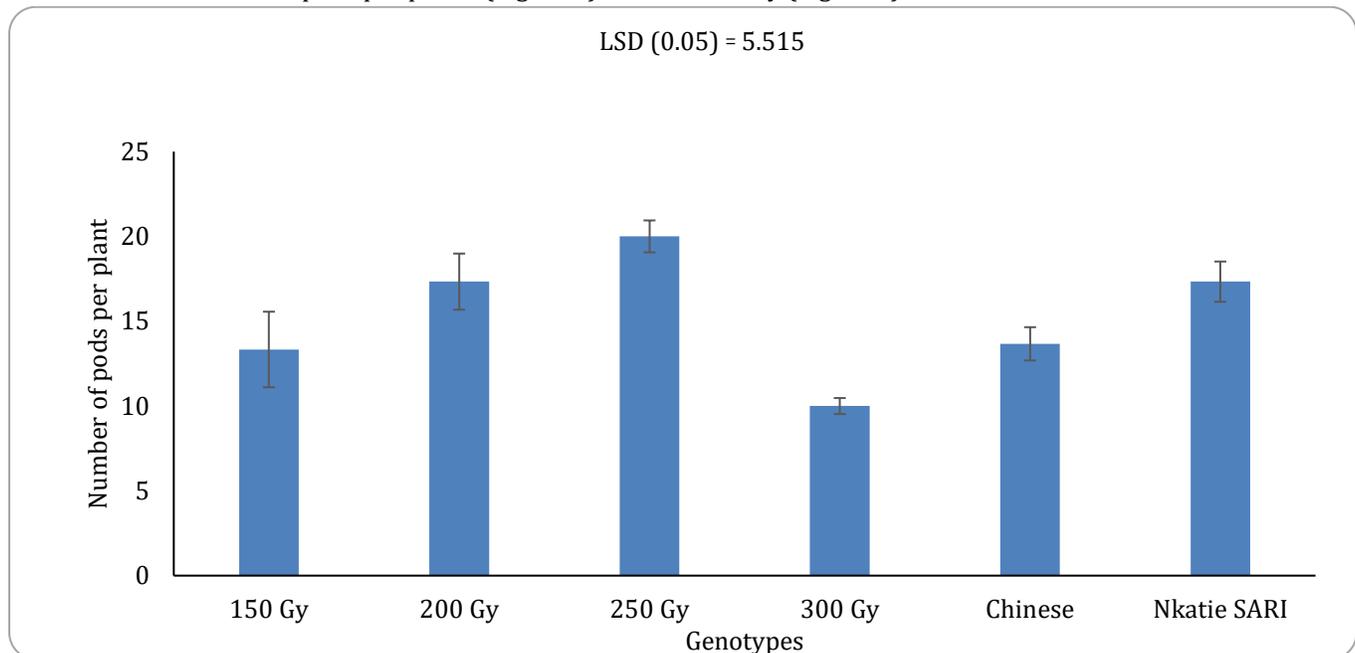


Figure 2. The influence of diseases incidences on groundnut pods per plant. Error bars represent standard error of means (SEM).

**Total grain yield:** The total grain yield was significantly different ( $P < .001$ ) among the genotypes. The highest grain yield was recorded by the 250 Gy, followed by the 200 Gy (Figure 3). The controls (Chinese and Nkatie SARI) had the lowest grain yield. The grain yield of the 250 Gy was

significantly different from that of the 150 Gy, 300 Gy and the controls (Chinese and Nkatie SARI). There was also a difference in grain yield between the 200 Gy and the 250 Gy. Again, the grain yield of the 200 Gy was also significantly different from the 150 Gy, 300 Gy and the controls (Chinese and Nkatie SARI).

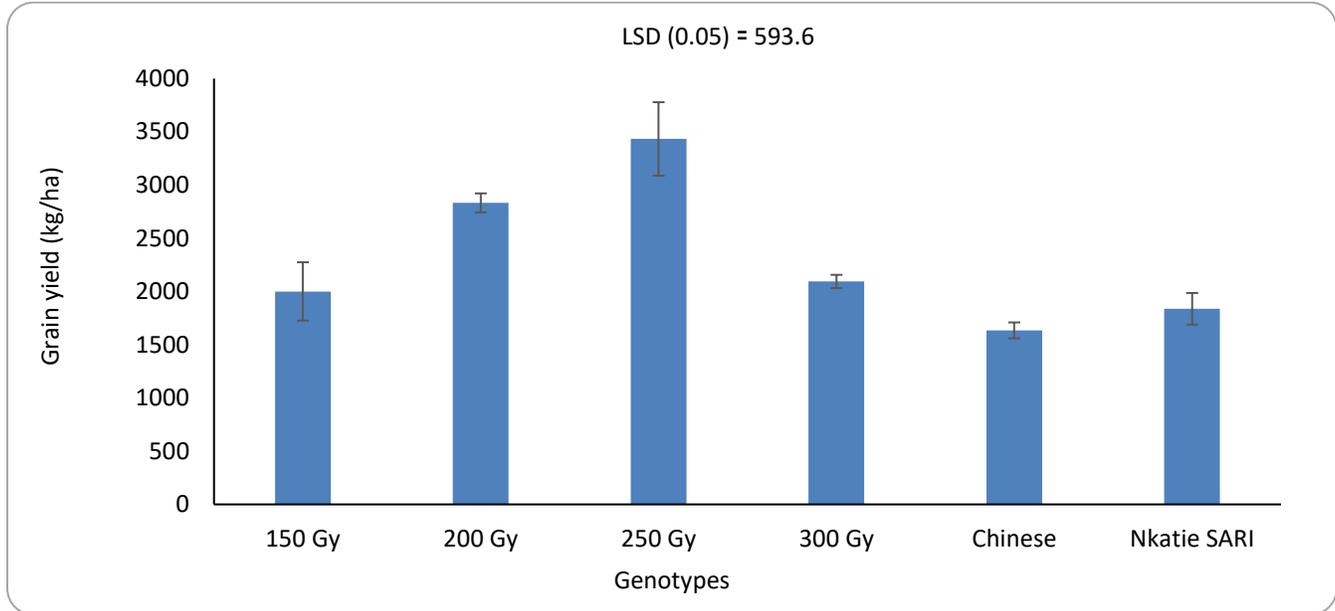


Figure 3: The influence of disease incidence on total grain yield. Error bars represent standard error of means (SEM)

**Correlations between disease severity scores, number of pods per plant and yield:** The results of correlation analysis among the traits studied are presented in Table 4. There were negative correlation between early leaf spot and groundnut rosette disease

and number of pods per plant. However, there was a positive correlation between the late leaf spot disease and pods per plant (Table 4). Also, the late leaf spot and rosette showed negative correlations with yield (Table 4).

Table 4: Correlations between disease severity scores, pod number per plant and yield

	Early leaf spot	Late leaf spot	Rosette	Pod no./plant	Grain yield
Early leaf spot	1				
Late leaf spot	0.224981572	1			
Rosette score	0.649062259	-0.022504562	1		
Pod per plant	-0.159177948	0.702117634	-0.482973	1	
Yield (kg/ha.)	0.255499032	-0.304938965	-0.411645	-0.04989	1

**DISCUSSION**

The study demonstrated that mutant genotypes 250 Gy recorded the least incidence of early leaf spot, late leaf spot and groundnut rosette diseases. Thus, only a few number of groundnut plants from these mutant genotypes showed the groundnut rosette disease symptoms. This means that even resistant cultivars are not completely immune to the rosette virus. The result is in line with Nigam *et al.* (2012) who reported that resistant varieties were not totally immune to the rosette virus disease with few plants showing mild

symptoms. However, the highest incidence of the early leaf spot, late leaf spot and groundnut rosette disease was registered by the Chinese and the Nkatie SARI (Figure 1). These two varieties were severely affected, leaves were distorted with multiple yellow to brown spots with halos, and few died as a result. In another study, Ngegba *et al.* (2017) reported high and moderate early and late leaf spot incidences in the ICIAR-19BT and NGB01293 and NGB00157and NGB01294 groundnut accessions, respectively. In the present study, though the diseases were present in the mutant genotypes, the

spread of their symptoms were relatively low as compared to those of the controls. Mutant genotype 250 Gy was resistant to early leaf spot and late leaf spot disease compared with the other mutant genotypes, which showed moderate resistance. This might be possibly due to genetic factor, as these genotypes (200 Gy and 250 Gy) were exposed to different doses of gamma radiations, altered their genetic constitutions, given rise to high resistant capacities against the diseases.

The severity of the groundnut rosette disease was high on the field and the genotypes exhibited variable level of resistance from 45 to 85 days after planting (Table 3). For instance, mutant genotypes 200 Gy and the 250 Gy were resistant to the groundnut rosette virus disease. This conforms to earlier reports that, severely infected rosette groundnut plants became stunted in growth, assumed bushy appearance as a result of persistent reduced internodes lengths and shrink or malformed leaves (A'brook, 2007). Bwala *et al.* (2019) also reported that the Samnut-10, Samnut-21 and Samnut-22 genotypes of groundnut were resistant to the rosette virus disease(s) when screened for resistance. The 150 Gy and the 300 Gy were also moderately resistant (Table 3). Appiah *et al.* (2016) reported similar findings under a field trial in Ghana, where some groundnut cultivars showed moderately resistant to resistant response to rosette disease.

The study revealed that genotype 250 Gy recorded the highest number of pods per plant which was significantly different from all the other genotypes and the controls. The result is in conformity to that of Alidu *et al.* (2019) who reported that genotype F-Mix had a higher number of pods per plant than the already existing groundnut cultivars in Northern Ghana. Mutant genotype 300 Gy had the lowest pods per plant, which might be attributed to the influence of the dose of the radiation on the genes of that genotype. The decrease in pods per plant varied marginally among the genotypes based on the degree of the severity of the disease(s). This is in line with Waliyar *et al.* (2000) earlier report that, the defoliation by foliar diseases such as early and late leaf spots diseases distorts the leaf cells and disrupts the processes of photosynthesis leading to reduction in pod yield. Number of pods per plant also correlated negatively with the rosette and early leaf spot diseases, as shown in Table 4, which means the increase in early leaf spot and rosette disease severity lead to decrease in

number of pods per plant.

The combined variable effects of the early leaf spot, late leaf spot and groundnut rosette diseases significantly influenced the total yield per hectare (Figure 3). Mutant genotype 250 Gy which was resistant against the three major groundnut diseases recorded the highest grain yield, followed by the 200 Gy which showed moderate resistance to ELS and LLS diseases. The Chinese and the Nkatie SARI varieties recorded low yields which might be attributed to inhibition of photosynthetic processes due to the intense reduction in leaf area and defoliation by the diseases. This is line with Singh and Shukla (2009) who reported a decrease in chlorophyll composition in disease affected plants. Most of the plants which were severely affected by the diseases did not produce pods. And this is consistent with earlier reports that, foliar diseases of groundnut such as rosette can cause an important yield loss in groundnut up to 100% (Dwivedi, *et al.*, 2003). Both the groundnut rosette disease and the late leaf spot disease showed negative correlations with grain yield (Table 4). Thus, as severities of the groundnut rosette disease and late leaf spot disease increases, the grain yield decreases. The result is in agreement to that of Mohammed *et al.* (2018) who reported significant negative correlations between the late leaf spot, rosette disease and yield of groundnut.

#### CONCLUSION

The mutant genotypes showed some levels of resistance against all the three major groundnut diseases. Mutant genotype 250 Gy was found to be resistant against all the three major diseases studied while genotype 150 Gy and 300 Gy showed moderate level of resistance. However, the 200 Gy was resistant to groundnut rosette disease. Mutant genotypes 150 Gy and 300 Gy were moderately resistant against the early leaf spot, late leaf spot and groundnut rosette disease and are likely to succumb to these diseases under high disease incidence. But the control (Chinese and Nkatie SARI) varieties were found to be susceptible to groundnut rosette virus disease. The correlation between late leaf spot and rosette diseases and yield parameters showed decrease in pods per plant and yield of groundnut as the severity of the disease increases. Mutant genotype 250 Gy can be used in further analytical breeding programs to identify and evaluate specific responsible genes at molecular level.

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Frederick Kankam	:	Design research, manuscript write up.
Kwame Y. Kojo	:	Conduct research and write up.
Isaac K. Addai	:	Help in conducting research and write up.