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SYSTEMATIC EVALUATION OF GROUNDNUT GENOTYPES FOR RESISTANCE TO CERCOSPORA LEAF SPOT DISEASE

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ABSTRACT

The present study assessed peanut genotypes for resistance to Cercospora leaf spot (CLS), with a focus on disease severity and defoliation across different growth stages. Based on Percent Incidence Data (PID) and defoliation scores, genotypes were classified as susceptible, moderately resistant, or resistant. Disease progression was measured through the Area under the Disease Progress Curve (AUDPC), revealing significant variation in resistance among the genotypes. Most genotypes, including 21CG001, 21CG002, 21CG003, 21CG004, 21CG006, ATTOCK 19, and 20AK012, exhibited high PID and defoliation levels, with AUDPC values indicating susceptibility. Their PID ranged from 23.96 to 31.72 at 50 days after sowing (DAS) and from 44.84 to 56.97 at 70 DAS. Genotypes 21CG005, 20AK004, and ATTOCK 19 showed the highest PID and defoliation levels, with AUDPC values over 320, categorizing them as highly susceptible. Conversely, genotypes 21CG007, 21CG008, 20AK001, and 20AK010 demonstrated the lowest PID, minimal defoliation, and AUDPC values below 250, indicating strong resistance. In vitro trials further highlighted variability in lesion characteristics, with genotypes 20AK012, BARI 16, and 20AK010 showing the most severe symptoms, including rough lesion textures and types ranging from minute chlorotic spots to mature black lesions. Overall, genotypes 21CG007, 21CG008, 20AK001, and 20AK010 exhibited high resistance, while ATTOCK 19, 20AK004, and 21CG005 were highly susceptible. These findings underscore the importance of selecting resistant genotypes for effective CLS management in peanut cultivation.

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INTRODUCTION

The cultivation of peanut (*Arachis hypogaea* L.) holds global significance, ranking as the fourth most important oilseed crop and playing a crucial role in sustaining the rural economy (Zanjare *et al.*, 2023). Peanut production is vital to both traditional farming practices and modern commercial systems across diverse regions (Nautiyal and Mejia, 2002). Grown in approximately 114 tropical and subtropical countries, peanuts serve a multitude of purposes, including human nutrition, livestock forage, and oilseed extraction, emphasizing its central role in global food security and agricultural industries.

With a global cultivation area of 29.55 million hectares, peanuts yield an impressive annual production of 44.5 million metric tons, translating to an average productivity of approximately 1.51 metric tons per

hectare (FAO, 2023). This extensive cultivation is concentrated in major peanut-producing countries such as India, China, the United States, Nigeria, and Sudan, which collectively contribute significantly to the global output of the crop. The adaptability of peanuts to various agronomic environments highlights its importance in diverse agricultural landscapes, making it a key component in the search to meet the growing food and economic needs of a rapidly expanding global population (ICRISAT, 2023).

Peanut production in Pakistan plays a vital role in the agricultural sector, particularly in the arid and semi-arid regions where it serves as a significant cash crop. It is mainly cultivated in Punjab, which contributes the largest share of production.

Peanut cultivation in Pakistan is significantly hindered by various biotic constraints that adversely affect crop yield and quality. Peanut crops are vulnerable to attacks by fungi, nematodes, aphids, and thrips, which not only directly damage the plants but also act as vectors for viral diseases. Among the most severe challenges are fungal diseases, particularly early leaf spots (ELS) and late leaf spots (LLS) caused by *Cercospora arachidicola* and *C. personata*, which lead to substantial defoliation and yield losses.

The prevalence of these pests and pathogens is aggravated by the lack of resistant peanut varieties and inadequate pest management strategies. Farmers often rely on chemical control measures, which can be costly and environmentally detrimental, yet insufficient to fully mitigate these biotic stresses.

These pathogens reduce yields by up to 70%. This substantial yield loss poses a significant challenge to realizing the full potential of the crop (Kankam *et al.*, 2022). Addressing the yield gap is critical for enhancing agricultural productivity.

Recent research emphasizes the importance of identifying and developing disease-resistant groundnut varieties to reduce reliance on harmful chemical treatments. This approach not only mitigates environmental impact but also promotes sustainable agricultural practices (Lobell *et al.*, 2009; Van Ittersum *et al.*, 2013).

The primary objective of the current study was to systematically evaluate and identify groundnut genotypes with resistance to early leaf spot and late leaf spot diseases. By examining genotype responses to these diseases under the specific environmental conditions of the Pothwar region, the study aimed to provide valuable understandings for selecting and breeding groundnut varieties tailored to this area. The data on disease severity collected during the study will be instrumental in identifying potential sources of resistance, thereby guiding future breeding programs. Ultimately, this work aimed to develop groundnut varieties with enhanced resistance to ELS and LLS, contributing to improved yield stability and agricultural resilience in the region.

MATERIALS AND METHODS

Collection of Seeds

Seeds of the selected genotypes (Table 1) were obtained from two sources viz. the Groundnut Section, Barani Agricultural Research Institute, Chakwal, and the Groundnut Research Station, Attock, Pakistan.

Time and Location

Field screening was conducted during the kharif season of 2023 at the Barani Agricultural Research Institute, Chakwal, situated at 72° longitude, 32° latitude, and 575 meters above mean sea level. This site and timing were selected as they represent the natural conditions for groundnut cultivation, where early leaf spot (ELS) and late leaf spot (LLS) diseases are prevalent.

Method of Sowing

The test lines were assessed under inoculated conditions using the "Infector Row Technique" (Acheampong *et al.*, 2024). Genotypes were sown on broad beds with four rows per bed. This method, recommended for peanut cultivation, offers several benefits, including improved water drainage, enhanced soil aeration, and better root growth (Paul, 2020). The elevated bed design increases surface area compared to conventional flat cultivation, while the four-row planting pattern optimizes space utilization and enhances farming efficiency.

Agronomic Practices Employed

Standard agronomic practices were meticulously followed, including the application of 60 kg of P_2O_5 as a basal fertilizer. Pendimethalin was applied at a rate of 1 kg active ingredient per hectare to control weeds. Irrigation commenced immediately after planting and was adjusted according to the moisture requirements of the crop.

Interestingly, no disease symptoms were observed during the post-rainy season, eliminating the need for specific disease management interventions. A calculated application of calcium sulfate dihydrate at 400 kg per hectare was administered at the peak of flowering.

Preventive measures were taken to protect the crop from insect infestations; however, no targeted measures were applied to mitigate foliar fungal diseases.

Screening of Genotypic Resistance to Leaf Spot Disease

***In vitro* Screening**

In a controlled experiment designed to screen genotypes for resistance to leaf spot disease, plastic pots (22.5 cm × 22.5 cm) filled with a pasteurized mixture of soil, sand, and compost in a 2:1:1 ratio were used. Five seeds were sown in each pot, and three healthy seedlings were retained after emergence. Thirty-day-old potted plants were inoculated by spraying a standard conidial suspension (2×10^4 conidia per ml) using a small garden hand sprayer over three consecutive late hours. The inoculated plants were covered with polythene bags to maintain moisture. Control plants were kept separate for comparison. This controlled setup ensures that differences in disease progression are attributed to genetic variation rather than environmental factors, providing reliable understandings for resistance levels.

***In vivo* Screening**

For the *in vivo* screening, infector rows were maintained after every four broad beds and along the trial boundaries to create optimal disease pressure. This arrangement ensures the reliability of the generated data. A cyclone spore collector was used to collect conidial suspensions of *C. arachidicola* and *C. personata* from naturally infected leaves of a susceptible cultivar. The collected inoculum was stored at -20°C. The susceptible peanut cultivar was sown in polybags in the greenhouse two weeks prior to the field trial. Thirty-five-day-old seedlings were artificially inoculated with conidia of ELS and LLS (5×10^4 spores ml^{-1}) using a spore solution mixed with 0.05% Tween 20 as a surfactant.

Water was sprayed on and around the inoculated plants and were covered during night for one week to maintain high humidity (95%). Infected polybag plants were then transplanted into the infector rows of the trial 50 days after sowing. Conidia of the leaf spot pathogens were sprayed on the infector rows at the same concentration. Sprinkler irrigation was provided for 30 min daily for one month starting from the day of field inoculation to enhance disease conditions for effective screening.

Disease Assessment

The Percent Disease Index (PDI) was calculated using the conventional methodology outlined by McKinney (1923). The formula used for PDI is the sum of all disease ratings, divided by the total number of ratings, multiplied by the maximum rating, and then multiplied by 100. Disease assessments were conducted at 10-day intervals following inoculation (DAI).

The severity evaluations involved a detailed examination of compound leaves, sampled from various heights along the main stem to account for potential variation in disease manifestation throughout the plant. This systematic approach provided a comprehensive understanding of genotype-specific responses to leaf spot at different stages post-inoculation. A 1-9 scale was used for assessing disease severity, offering a thorough and meticulously detailed evaluation, with precise quantification of leaf spot incidence. This scoring system, proposed by Subrahmanyam *et al.* (1995), ensures consistency and comparability of severity data across genotypes.

Initial data was recorded at 10-day intervals after inoculation, with subsequent intervals chosen strategically to capture the dynamic progression of leaf spot development over time. By considering the position of compound leaves, chosen time intervals, and a well-established severity scale, this methodological rigor provides reliable and informative data on resistance or susceptibility of groundnut genotypes to early and late leaf spot diseases.

The Area under the Disease Progress Curve (AUDPC) values were calculated directly from empirical data and estimated using a defined mathematical formula. These calculated AUDPC values were then compared with those obtained through the equation. The comparison involved plotting and ranking the calculated values against the estimated ones, resulting in a remarkably high level of correspondence. This correspondence was quantified using Spearman's Rank Correlation, which produced coefficients of 0.9879 for the Karamu trial and 0.9515 for the Oroua trial. These strong correlation coefficients highlight the effectiveness of estimating AUDPC values from a limited set of data points. The findings suggest that a concise set of observations can provide data comparable to that obtained from more extensive, repeated assessments.

RESULTS

Categorization of Genotypes based on Disease Severity and Defoliation across Growth Stages

The genotypes were assessed for disease severity and

defoliation at different growth stages, with results categorized as susceptible, moderately resistant, or resistant based on their percent incidence data (PID) and defoliation scores. Overall, the resistance of genotypes varied significantly, with some showing high susceptibility to the disease while others exhibited strong resistance. Most of the genotypes, including 21CG001, 21CG002, 21CG003, 21CG004, 21CG006, ATTOCK 19, and 20AK012, showed high PID values and significant defoliation across growth stages, with AUDPC values indicating high susceptibility. Their PID ranged from 23.96 to 31.72 at 50 DAS and from 44.84 to 56.97 at 70 DAS, with AUDPC values between 301.45 and 345.97 (Table 1). Some genotypes namely 21CG005, 20AK004, and ATTOCK 19 exhibited the highest PID values and severe defoliation, with AUDPC values of 345.97, 341.35, and 334.36, respectively. They were categorized as highly susceptible. Two genotypes (BARI 16 and 20AK011) demonstrated lower PID values and moderate defoliation, with AUDPC values of 251.97 and 266.72, respectively, indicating moderate resistance. On the other hand, the genotypes such as 21CG007, 21CG008, 20AK001, and 20AK010 had the lowest PID values and minimal defoliation, with AUDPC values ranging from 226.86 to 231.89. These genotypes were categorized as resistant (Table 1).

***In vitro* Disease Symptoms and Lesion Characteristics**

A summary of disease symptoms and lesion characteristics for various genotypes assessed in an *in vitro* trial is given in Table 2. The characteristics measured include the number of minute chlorotic spots, brown lesions, mature black lesions, and roughness of lesions on the lower surface.

In case of minute chlorotic spots, genotypes varied from 10 to 21 spots. The genotype 21CG005 had the fewest minute chlorotic spots (10), while 20AK010 had the most (21). The count in case of brown lesions ranged from 12 to 26 lesions. Genotype 21CG005 had the lowest number (12), and genotype 20AK010 had the highest number (26) of brown lesions. The number of mature black lesions ranged from 14 to 32. The minimum was observed in genotype 21CG005 (14), and the maximum was in genotype 20AK010 (32). The roughness of lesions on lower surface was assessed qualitatively and ranged from 27 to 43. Genotype 21CG005, ATTOCK 19 and 20AK004 did not show roughness, while genotype 20AK010 exhibited the highest roughness (43) followed by 21CG008 (41).

On the whole, genotypes 20AK012, BARI 16, 20AK010, 21CG007 and 21CG008 displayed the most severe symptoms across all categories, with higher counts of chlorotic spots, brown and mature black lesions, and rougher lesions on the lower surface. On the other hand, genotypes 20AK004, ATTOCK 19 and 21CG005 showed the lowest counts for all observed lesion characteristics (Table 2).

Categorization of Peanut Genotypes against CLS

Table 3 presents the results of peanut genotypes for their resistance to Cercospora leaf spot. The genotypes were categorized based on the AUDPC, a measure of disease severity over time.

Four genotypes (21CG007, 21CG008, 20AK001, and 20AK010) showed high resistance to CLS with AUDPC values below 250. Two genotypes (20AK011 and BARI 16) exhibited moderate resistance with AUDPC values between 250 and 270. Six genotypes (21CG001, 21CG002, 21CG003, 21CG004, 21CG006, and 20AK012) were susceptible with AUDPC values ranging from 271 to 320. Three genotypes (ATTOCK 19, 20AK004, and 21CG005) were highly susceptible with AUDPC values exceeding 320 (Table 3).

DISCUSSION

Leaf spot causes premature leaf drop and fallen leaves with lesions serve as the primary inoculum for spores if peanuts are cultivated in the same or adjacent fields in the following season (Pal *et al.*, 2014). Due to the economic significance of this disease, scientists have consistently pursued strategies to reduce its impact (Denwar *et al.*, 2021). These strategies include chemical control (Kaur *et al.*, 2024), biocontrol (Ahmed *et al.*, 2023; Hasnain *et al.*, 2024; Iqbal *et al.*, 2014; Shahbaz *et al.*, 2023), crop management techniques (Richard *et al.*, 2022; Woo *et al.*, 2022), and incorporating heritable resistance (Mugisa *et al.*, 2016).

Although fungicides are widely used for managing leaf spot (Manzoor *et al.*, 2024), they pose significant risks to society (Admasu *et al.*, 2024; Iqbal and Mukhtar, 2020; Morio *et al.*, 2024). Chemicals containing active compounds like methyl bromide and strobilurin have harmful effects on non-target organisms and can contaminate both groundwater and surface water sources and has led to growing concerns about their use (Bulathsinghala and Shaw, 2014; Cullen *et al.*, 2019; Feng *et al.*, 2020).

Table 1. Data of percent incidence of CLS and defoliation under field conditions.

Genotypes	PID	PID	Defoliation	PID	Defoliation		Scale	Category
	50 DAS	70 DAS	70 DAS	90 DAS	90 DAS	AUDPC		
21CG001	25.33	47.25	27.01	52. 87	33.59	310.78	7	Susceptible
21CG002	23.96	44.84	25.53	53.03	34.58	301.45	7	Susceptible
21CG003	27.58	48.46	27.6	56.97	37.38	314.38	7	Susceptible
21CG004	24.05	45.35	25.83	53.73	34.98	312.66	7	Susceptible
21CG005	31.72	45.2	31.44	85.14	42.58	345.97	9	Highly Susceptible
21CG006	29	48.98	27.89	56.67	37.78	316.33	7	Susceptible
21CG007	2.7	4.28	24.65	8.91	33.38	236.08	3	Resistant
21CG008	2.08	3.65	22.58	6.97	30.59	226.86	3	Resistant
ATTOCK 19	28.89	40.39	26.42	65.15	35.78	334.36	8	Highly Susceptible
BARI 16	6.61	12.76	24.35	15.21	32.98	251.97	4	Moderately Resistant
20AK001	2.36	3.28	24.65	8.91	33.38	231.89	3	Resistant
20AK004	30.21	44.32	25.24	70.32	34.18	341.35	8	Highly Susceptible
20AK010	2.32	3.13	22.29	8.27	30.19	228.80	3	Resistant
20AK011	7.45	13.8	24.94	19.62	33.78	266.72	5	Moderately Resistant
20AK012	21.52	42.24	24.06	37.5	32.58	289.66	6	Susceptible

Table 2. Assessment of disease symptoms and lesion characteristics in various genotypes in *in vitro* trial.

Genotypes	Minute chlorotic spots	Brown lesions	Mature black lesions	Roughness of lesions on lower surface
21CG001	16	19	23	31
21CG002	17	20	24	33
21CG003	15	18	21	27
21CG004	17	20	24	32
21CG005	10	12	14	**
21CG006	11	13	16	27
21CG007	20	23	28	38
21CG008	20	25	31	41
ATTOCK 19	10	12	15	**
BARI 16	20	24	29	39
20AK001	20	23	28	37
20AK004	11	12	15	**
20AK010	21	26	32	43
20AK011	19	22	27	36
20AK012	20	24	29	39

Table 3. Categorization of Peanut Test Entries against CLS.

Scale	AUDPC	Reaction	Genotypes	Names of Genotypes
3 and < 3	< 250	Resistant	4	21CG007, 21CG008, 20AK001, 20AK010
4-5	250-270	Moderately Resistant	2	20AK011 ,BARI 16
6-7	271-320	Susceptible	6	21CG001, 21CG002, 21CG003, 21CG004, 21CG006, 20AK012,
8-9	>320	Highly Susceptible	3	ATTOCK 19, 20AK004, 21CG005

Employing leaf spot-resistant cultivars has proven to be an effective management strategy (Mohammed *et al.*, 2018). However, these varieties are not widely accessible to farmers, and resistance may be compromised by the emergence of new biotypes of *C. arachidicola* and *C. personatum* (Mohammed *et al.*, 2018).

Fungicides, while effective, are often too costly for small-scale farmers (Gonzales *et al.*, 2023). In contrast, breeding disease-resistant cultivars offers a more sustainable and environmentally friendly solution (Abebele and Zerihun, 2024; Afzal *et al.*, 2024; Dar *et al.*, 2024; Hussain *et al.*, 2024; Nigar *et al.*, 2024; Soomro *et al.*, 2024). In the present study, results from both field screening and pot culture experiments demonstrated that some groundnut genotypes, namely 21CG007, 21CG008, 20AK001, and 20AK010, exhibited a significantly slower progression of leaf spot disease compared to the susceptible cultivars 21CG001, 21CG002, 21CG003, 21CG004, 21CG006, ATTOCK 19, 20AK004, and 21CG005. The susceptibility of the latter cultivars was evidenced by a notably high rate of disease progression. These findings indicate that while absolute resistance or immunity is not present in the cultivated genotypes, several groundnut lines exhibit a high degree of resistance. This level of resistance is comparable to the slow rusting reaction observed in cereal crops (Wilcoxson, 1981).

In *in vitro* inoculations, genotypes were carefully monitored for the sequential development of leaf spot symptoms. The appearance of morphological symptoms was tracked over time for both resistant and susceptible genotypes. Chlorotic spots became visible on susceptible leaves after nine to ten days, while they did not appear on resistant genotypes until 14 to 19 days. The progression to distinct dark brown lesions, characteristic of leaf spot, occurred 15 to 16 days after inoculation in susceptible genotypes, compared to 21 to 28 days in resistant genotypes. Moreover, lesions on resistant genotypes were less rough due to sporulation than those on susceptible genotypes. Notably, specific genotypes, such as 20CG007, 21CG008, and 20AK010, demonstrated prolonged resistance, with symptoms appearing up to 29 to 32 days post-inoculation.

Previous studies by researchers such as Gopal *et al.* (1994) identified specific genotypes, including R 8972, as highly resistant to leaf spot diseases in groundnuts. Building on these findings, subsequent hybridization efforts involving both resistant and susceptible cultivars were undertaken to develop high-yielding, disease-resistant varieties. Similar methodologies and achievements have been documented by Bera and Ghose (1999), Jeger and Viljanen-Rollinson (2001), Lobell *et al.* (2009), Thakur *et al.* (2012), Narh *et al.* (2014), Gaikpa *et al.* (2015), Méndez-Natera *et al.* (2016), Asad *et al.* (2017), Zongo *et al.* (2017), Houshyarfard and Padasht Dahkai (2018), and Zanjare *et al.* (2023). These collective findings have significantly contributed to the understanding of disease resistance mechanisms in groundnuts and offer valuable understandings for the genetic improvement of the crop.

Genetic erosion and the polyploid nature of groundnuts present challenges to their improvement (Janila *et al.*, 2013). Identifying genotypes with varying levels of resistance to Cercospora leaf spot provides a critical resource for breeding programs aimed at developing resilient groundnut varieties (Kongola, 2018). This information helps breeders select parent plants with moderate to high resistance for crossing programs, thereby enhancing genetic diversity and disease resistance in new cultivars (Dey *et al.*, 2024). Integrating these resistant genotypes into breeding strategies can lead to varieties that not only withstand disease pressures but also maintain or improve yield, contributing to sustainable agriculture and food security (Denwar *et al.*, 2021).

Ongoing genetic research highlights the need for continuous screening and evaluation of groundnut germplasm as new pathogen strains emerge. Developing cultivars with durable resistance remains crucial, and the efforts of pioneering scientists have laid the foundation for future advancements in groundnut breeding, emphasizing the importance of innovation and collaboration in addressing these challenges.

CONCLUSION

In this study, peanut genotypes were assessed for their resistance to Cercospora leaf spot (CLS) through the evaluation of disease severity, defoliation, and lesion characteristics. Significant variability in resistance was observed among the genotypes, with some showing strong resistance, while others were highly susceptible. Genotypes 21CG007, 21CG008, 20AK001, and 20AK010 consistently demonstrated low Percent Incidence Data (PID), minimal defoliation, and low Area under the Disease Progress Curve (AUDPC) values, indicating strong resistance to CLS. In contrast, genotypes such as 21CG005, 20AK004, and ATTOCK 19 exhibited high PID, defoliation, and AUDPC values, categorizing them as highly susceptible. The identification of resistant genotypes is crucial for integrating disease-resistant varieties into peanut breeding programs and developing effective CLS management strategies.

FUTURE DIRECTIONS

Future studies should focus on further validating the resistance of promising genotypes under diverse agro-climatic conditions and across multiple growing seasons. Molecular and genetic analyses can help identify resistance markers, facilitating the breeding of CLS-resistant peanut varieties. Additionally, integrating resistant genotypes with other management practices, such as fungicide applications and cultural control methods, should be explored to enhance overall disease management. Long-term monitoring of CLS resistance and the potential for resistance breakdown over time will also be essential to ensure sustained protection against this pathogen.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS CONTRIBUTION

SM, TM and AA conceived the idea; SM and AA designed the study, conducted the field trial, collected and compiled the data; SM wrote the first draft; TM and AA supervised the work, oversaw the planning, execution, and interpretation of results, ensuring the project adhered to high scientific standards and proofread the manuscript; MAK provided critical evaluation and expert advice at various stages of the project.

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