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ORIGINAL RESEARCH ARTICLE

# Screening of blackgram (*Vigna mungo*) genotypes for resistance to cercospora leaf spot and yield performance in Nepal

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**ABSTRACT**

This study screened 12 black gram (*Vigna mungo* L.) genotypes for resistance to Cercospora leaf spot (CLS) caused by *Cercospora canescens*. Conducted at the research plot of the Mid-West Academy and Research Institute, Tulsipur, Dang, the experiment utilized a randomized complete block design with three replications from September 2022 to January 2023. Disease severity was evaluated at 40, 47, and 54 days after sowing (DAS), with scoring based on the percentage of infected leaf area at 7-day intervals. Key disease metrics—incidence, severity, mean area under the disease progress curve (AUDPC), and yield—were calculated. Results indicated no significant difference in CLS incidence among genotypes. However, significant differences in disease severity were noted at 40, 47, and 54 DAS, with AUDPC values differing across genotypes. Based on mean AUDPC values, 10 genotypes were categorized as moderately resistant, while BLG 0066-1-1 and BLG 0035-1 were moderately susceptible. BLG 0035-1 had the highest AUDPC (324.1), and BLG 0069-1 had the lowest (175). Yield varied significantly, with the highest yield (799 kg/ha) recorded for BLG 0068-2 and the lowest (495 kg/ha) for BLG 0066-1. These findings support breeding efforts targeting CLS resistance and enhanced yield in black gram.



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## INTRODUCTION

Blackgram (*Vigna mungo* L.), also known as urd bean or black matpe, is a staple legume in South Asia, including Nepal, where it is widely consumed as "maas ko daal" or "kalo daal," a dish served with rice and curry. In Nepal, blackgram cultivation spans 23,056 hectares, producing approximately 20,440 metric tons with an average yield of 887 kg/ha (MoAD, 2022). Leguminous are known for its nutrient content and nitrogen-fixing ability, moong bean, cowpea and blackgram enhances soil fertility, allowing it to thrive in diverse climates (Kumar & Chopra, 2012; Srivastava *et al*., 2017). However, biotic factors like disease and abiotic stresses significantly hamper blackgram production, leading to considerable yield losses. Among the major biotic threats, Cercospora Leaf Spot (CLS) disease, caused by the fungal pathogen *Cercospora canescens*, poses a substantial risk to blackgram in tropical and humid regions across South Asia, including Nepal, India, Bangladesh, Indonesia, and the Philippines (Gupta *et al*., 2013; Kumar *et al*., 2014). First reported in Delhi, India (Munjal *et al*., 1962), CLS disease can lead to yield losses as high as 96% under severe epiphytotic conditions Kaur *et al*., 2004. The pathogen infects blackgram primarily during the flowering and pod-filling stages, causing necrotic lesions that spread rapidly, resulting in premature defoliation and reduced seed development, with yield losses reported between 46% and 61% (Chand *et al*., 2015).

Despite the importance of blackgram, Nepalese farmers often lack access to high-quality, resistant seeds and scientific crop

management practices, leading to frequent CLS outbreaks. Control of CLS is typically attempted with fungicides, yet this approach often overlooks optimal application strategies, posing health, ecological, and resistance risks. To date, breeding for CLS-resistant blackgram genotypes remains limited, and there is a lack of comprehensive studies comparing genotype resistance levels under Nepalese field conditions. This research aims to address this gap by screening various blackgram genotypes for CLS resistance and yield performance, with a focus on identifying genotypes that combine disease resistance and high yield (Kaur *et al.*, 2004; Chand *et al.*, 2015). This study's findings will support breeding programs targeting CLS-resistant and high-yielding blackgram varieties suitable for Nepalese growing conditions. Specifically, we aim to: (a) assess CLS incidence, (b) evaluate disease severity across genotypes, and (c) estimate the mean area under the disease progress curve (AUDPC) and yield performance.

## MATERIALS AND METHODS

### Study site

The experiment was conducted during the summer season of 2021 at the field site and pathology lab of the Institute of Agriculture and Animal Sciences (IAAS), MARICOLS, Dang district, Nepal. The location (27.9904°N, 82.3018°E, 725 m above mean sea level) falls within the inner Terai region of Nepal, with a subtropical climate.

### Experimental details

Twelve blackgram (*Vigna mungo* L.) genotypes were collected from the Grain Legume Research Program, Khajura, Banke, Nepal. The experiment's layout was in a Randomized Complete Block Design (RCBD) with replications done thrice. Each genotype was allocated to a 2 m × 1 m plot, with a row-to-row spacing of 25 cm. The gross experimental area was 84 m^2^, and the layout consisted of three rows, each containing 12 plots. Well-decomposed farmyard manure (FYM) at 15 kg/m^2^ was applied during field preparation, and inorganic fertilizers (urea, diammonium phosphate, and potash) were supplied at the recommended dose of 20:40:20 N: P: K kg/ha.

### Cultivation practices

Land preparation was carried out on September 11, where plowing thrice, planking, and then leveling was done. Raised beds of 10-15 cm height were prepared for sowing to avoid waterlogging. Seeds were sown on the raised beds by the line sowing method at a rate of 6 g per plot. Hand weeding was performed twice, on the 13th-14th and 39th-40th days after sowing (DAS). Chlorpyrifos and cypermethrin (2ml per liter) were sprayed systematically on October 24, 2021 to control hairy caterpillars. No irrigation was provided due to sufficient rainfall during the crop growth period.

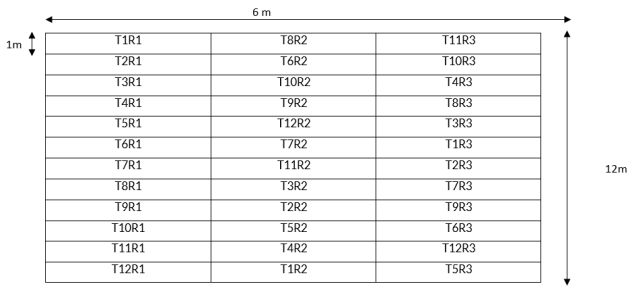


**Figure 1.** *Layout of the experimental field.*

**Table 1.** List of black gram genotypes used for the experiment.

| S. No. | Treatments | Symbol |
|---|---|---|
| 1 | Rampur mas | T1 |
| 2 | BLG 0068-2 | T2 |
| 3 | BLG 0076-2 | T3 |
| 4 | BLG 0069-1 | T4 |
| 5 | BLG 0061-2-2 | T5 |
| 6 | Khajura mas-1 | T6 |
| 7 | BLG 0066-1-1 | T7 |
| 8 | BLG 0072-1 | T8 |
| 9 | BLG 0072-1 | T9 |
| 10 | BLG 0035-1 | T10 |
| 11 | Shekhar | T11 |
| 12 | BLG 0041-1 | T12 |

| Grade | Percentage infection | Reaction |
|---|---|---|
| 1 | No infection on leaves | Resistant (R) |
| 2 | 0.1% to 5% infection on the leaf surface | Moderately resistant (MR) |
| 3 | 5.1% to 10% infection on the leaf surface | Moderately resistant (MR) |
| 4 | 10.1% to 15% infection on the leaf surface | Moderately susceptible (MS) |
| 5 | 15.1% to 30% infection on the leaf surface | Moderately susceptible (MS) |
| 6 | 30.1% to 40% infection on the leaf surface | Susceptible (S) |
| 7 | 40.1% to 50% infection on the leaf surface | Highly susceptible (HS) |
| 8 | 50.1% to 75% infection on the leaf surface | Highly susceptible (HS) |
| 9 | Above 75% infection on the leaf surface | Highly susceptible (HS) |

### Disease incidence

The number of germinated plants per plot of the individual genotypes was counted. Once the disease appeared, the number of infected plants was counted to calculate the incidence of Cercospora leaf spot (CLS), using the following formula.

$$\text{Cercospora leaf spot incidence} = \frac{\text{No. of diseased plant}}{\text{No. of germinated plant per plot}} \times 100$$

### Disease scoring

Disease incidence was recorded after the appearance of the disease (3 times scoring was done at the interval of 7 days at 40 DAS, 47 DAS, and 54 DAS). These scorings were taken at 7 days intervals during the pod formation stage. The data obtained from the experiment were grouped into five categories as resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S), and highly susceptible (HS) types to determine the nature of genotypes. The score 1 was considered

as resistant whereas 2-3 was moderately resistant, 4-6 as moderately susceptible, 6 as susceptible, and 7-9 were considered highly susceptible. The disease scoring chart was given by (Alice & Nadarajan, 2007) is given as Table 2. Disease severity per plot was determined based on the recorded scores, calculated with the following formula.

$$\text{PDI} = \frac{\text{No. of observation assessed} \times \text{maximum disease rating}}{\text{Sum of individual disease ratings}} \times 100$$

### Estimation of the area under disease progressive curve (AUDPC)

The area under the disease progress curve (AUDPC) was calculated by summarizing the progress of disease severity. The progression of the epidemic, reflected by lesion count, diseased tissue area, or the number of infected plants, is represented by a curve known as disease progress curve, that shows epidemic over time and the area covered by this curve is known as AUDPC. AUDPC was calculated from disease severity values using the formula given by (Shaner & Finney, 1977) as well as Das *et al.* (1992).

$$AUDPC = \sum_{i=1}^{n-1} \left( \frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

Where,

Yi= disease severity on the first date

Ti= date on which the disease was scored

N= number of dates on which disease was scored.

### Statistical analysis

Data were tabulated and analyzed using M-STAT software. A 5% significance level ANOVA was used to evaluate differences among genotypes in terms of disease incidence, severity, AUDPC, and yield, with the least significant difference (LSD) test applied for mean separation. A regression analysis was performed to quantify the relationship between AUDPC and yield.

## RESULTS AND DISCUSSION

### Disease incidence

The analysis of variance (ANOVA) indicated no significant differences in Cercospora leaf spot (CLS) disease incidence among the twelve blackgram genotypes (P = 0.66), with values ranging from 6.3% (BLG 0066-1-1) to 10.3% (BLG 0035-1) and an overall mean of 7.7% (CV = 15%). This uniformity in incidence suggests that, although all genotypes were initially infected, they displayed similar susceptibility to primary CLS infection. These findings align with (Bhaskar, 2017), who observed minimal variance in CLS incidence across blackgram genotypes in comparable subtropical environments.

### Disease severity

Disease severity, however, varied significantly among genotypes (P < 0.01) across the scoring dates of 40, 47, and 54 days after sowing (DAS). At 40 DAS, BLG 0035-1 exhibited the highest severity (40.74%), followed by BLG 0066-1-1 (37.04%), whereas BLG 0069-1 and Shekhar displayed the lowest severity (18.52%). By 54 DAS, severity escalated, with BLG 0035-1 and BLG 0066-1-1 reaching a high of 55.55%, while Shekhar remained least affected (25.93%). The progression in disease severity over time could be attributed to differences in genetic resistance to CLS among genotypes, particularly under field conditions conducive to fungal spread. Recent studies have also highlighted that genotypic variation in disease severity correlates with a genotype's ability to resist secondary infection and limit fungal colonization on leaf surfaces. Such genetic variability emphasizes the importance of screening blackgram varieties in field settings to identify genotypes that inhibit disease progression effectively.

### Disease score, AUDPC and genotype reaction

There were notable differences in the mean disease score and the area under the disease progress curve (AUDPC) values among the genotypes (P < 0.01). Mean disease scores ranged from 2.1 in Shekhar to 4.3 in BLG 0035-1, while AUDPC values spanned from 168.5 (Shekhar) to 324.1 (BLG 0035-1). Based on these values, BLG 0066-1-1 and BLG 0035-1 were categorized as moderately susceptible, whereas the other ten genotypes, including Rampur Mas, BLG 0068-2, and Shekhar, were classified as moderately resistant to CLS. The categorization of these genotypes into susceptibility groups aligns with recent findings by (Adhikari *et al.*, 2023), who reported similar AUDPC variability in their assessment of blackgram resistance. The lower AUDPC and disease scores observed in genotypes like Shekhar and Rampur Mas suggest inherent mechanisms for controlling disease progression, potentially linked to factors such as cell wall reinforcement and biochemical defense pathways. These genotypic responses provide valuable insights for breeding programs aimed at enhancing resistance to CLS.

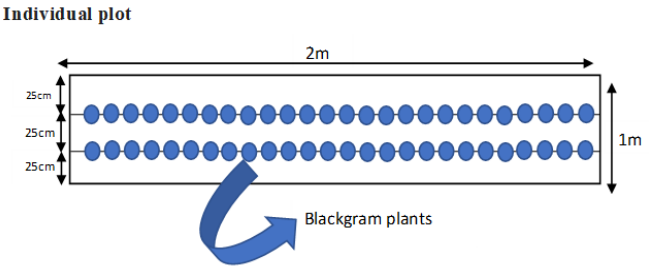


**Figure 2.** *Disease score chart.*

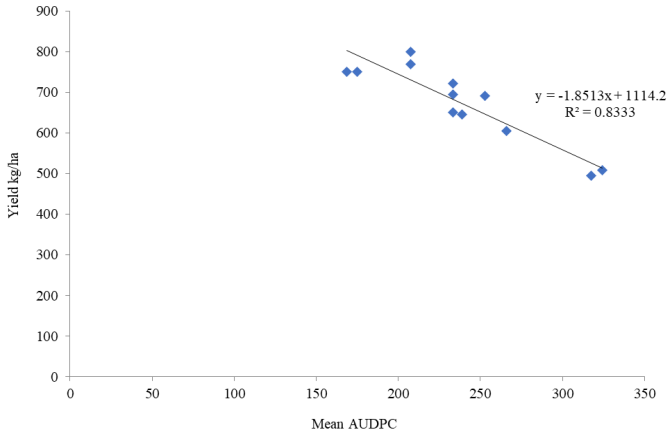


**Figure 3.** *Regression analysis between mean AUDPC and yield.*

**Table 2.** Cercospora leaf spot disease incidence of blackgram genotypes.

| S. No. | Treatments | Incidence % |
|---|---|---|
| 1 | Rampur Mas | 6.8 |
| 2 | BLG 0068-2 | 7.6 |
| 3 | BLG 0076-2 | 6.8 |
| 4 | BLG 0069-1 | 6.5 |
| 5 | BLG 0061-2-2 | 7.6 |
| 6 | Khajura Mas-1 | 7.5 |
| 7 | BLG 0066-1-1 | 6.3 |
| 8 | BLG 0068-1-1 | 7.2 |
| 9 | BLG 0072-1 | 9.7 |
| 10 | BLG 0035-1 | 10.3 |
| 11 | Shekhar | 8.4 |
| 12 | BLG 041-1 | 6.6 |
| Probability | | 0.66 |
| CV% | | 15 |
| LSD | | NS |

CV%: Coefficient of Variation, LSD: Least Significant Difference, NS: Non-significant.

### Yield performance and relationship with disease severity

Yield performance differed significantly among genotypes ($P < 0.01$), ranging from 495 kg/ha in BLG 0066-1-1 to 799 kg/ha in BLG 0068-2. High-yielding genotypes included Rampur Mas (769 kg/ha), BLG 0069-1 (750 kg/ha), and Shekhar (750 kg/ha). Regression analysis demonstrated a strong negative correlation ($R^2 = 0.833$) between mean AUDPC and yield, indicating that disease severity accounted for 83.3% of yield variability across genotypes. This negative correlation aligns with (Bhardwaj & Thakur, 2000), who similarly observed yield losses in blackgram due to CLS. The high AUDPC in BLG 0035-1 and BLG 0066-1-1, coupled with their low yields, underscores the detrimental impact of CLS on blackgram productivity. This effect is corroborated by recent studies (Chand *et al.*, 2015), which found that CLS significantly reduces seed filling and plant growth under conditions of high disease pressure.

**Table 3.** Disease severity of blackgram genotypes at different date of scoring.

| S. No. | Treatments | Disease severity | | |
|---|---|---|---|---|
| | | 40 DAS | 47 DAS | 54 DAS |
| 1 | Rampur Mas | 22.22 cd | 33.33 bc | 29.63 de |
| 2 | BLG 0068-2 | 25.93 cd | 29.63 bc | 33.33 cde |
| 3 | BLG 0076-2 | 25.93 cd | 40.74 a | 44.44 b |
| 4 | BLG 0069-1 | 18.52 d | 25.93 c | 29.63 de |
| 5 | BLG 0061-2-2 | 25.93 cd | 33.33 bc | 40.74 bc |
| 6 | Khajura Mas-1 | 29.63 bc | 33.33 bc | 37.04 bcd |
| 7 | BLG 0066-1-1 | 37.04 ab | 44.44 a | 55.55 a |
| 8 | BLG 0068-1-1 | 29.63 bc | 33.33 bc | 37.04 bcd |
| 9 | BLG 0072-1 | 29.63 bc | 37.04 ab | 40.74 bc |
| 10 | BLG 0035-1 | 40.74 a | 44.44 a | 55.55 a |
| 11 | Shekhar | 18.52 d | 25.93 c | 25.93 e |
| 12 | BLG 041-1 | 29.63 bc | 33.33 bc | 40.74 bc |
| SEm± | | 3.10 | 2.34 | 2.65 |
| CV% | | 19.38 | 11.76 | 11.75 |
| LSD | | 9.11** | 6.88** | 7.79** |

DAS: Days after sowing, CV%: Coefficient of Variation, LSD: Least Significant Difference Sem: Standard Error Mean, Means followed by the same letter in a column are not significantly different at 5% level of significance, **: Highly Significant.

**Table 4.** Mean disease score, mean AUDPC, and disease reaction of blackgram genotypes to Cercospora leaf spot disease.

| S. No. | Genotypes | Mean Disease score | Mean AUDPC | Reaction |
|---|---|---|---|---|
| 1 | Rampur Mas | 2.5 ab | 207.4 cd | Moderately Resistant (MR) |
| 2 | BLG 0068-2 | 2.6 ab | 207.4 cd | Moderately Resistant (MR) |
| 3 | BLG 0076-2 | 3.3 ab | 265.7 b | Moderately Resistant (MR) |
| 4 | BLG 0069-1 | 2.2 b | 175.0 d | Moderately Resistant (MR) |
| 5 | BLG 0061-2-2 | 3.0 ab | 233.3 bc | Moderately Resistant (MR) |
| 6 | Khajura Mas-1 | 3.0 ab | 233.3 bc | Moderately Resistant (MR) |
| 7 | BLG 0066-1-1 | 4.1 a | 317.6 a | Moderately Susceptible (MS) |
| 8 | BLG 0068-1-1 | 3.0 ab | 233.3 bc | Moderately Resistant (MR) |
| 9 | BLG 0072-1 | 3.2 ab | 252.8 b | Moderately Resistant (MR) |
| 10 | BLG 0035-1 | 4.3 a | 324.1 a | Moderately Susceptible (MS) |
| 11 | Shekhar | 2.1 b | 168.5 d | Moderately Resistant (MR) |
| 12 | BLG 041-1 | 3.1 ab | 239.8 bc | Moderately Resistant (MR) |
| SEm± | | 0.54 | 2.34 | |
| CV% | | 9.79 | 9.07 | |
| LSD | | 1.60 ** | 6.88** | |

AUDPC: Area Under Disease Progress Curve

**Table 5.** Mean AUDPC and yield of blackgram genotypes.

| S. No. | Genotypes | Mean AUDPC | Yield (kg/ha) |
|---|---|---|---|
| 1 | Rampur Mas | 207.4 cd | 769 a |
| 2 | BLG 0068-2 | 207.4 cd | 799 a |
| 3 | BLG 0076-2 | 265.7 b | 605 cd |
| 4 | BLG 0069-1 | 175.0 d | 750 ab |
| 5 | BLG 0061-2-2 | 233.3 bc | 650 bc |
| 6 | Khajura Mas-1 | 233.3 bc | 695 abc |
| 7 | BLG 0066-1-1 | 317.6 a | 495 e |
| 8 | BLG 0068-1-1 | 233.3 bc | 721 ab |
| 9 | BLG 0072-1 | 252.8 b | 691abc |
| 10 | BLG 0035-1 | 324.1 a | 509 de |
| 11 | Shekhar | 168.5 d | 750 ab |
| 12 | BLG 041-1 | 239.8 bc | 645 bc |
| SEm± | | 2.34 | 34.60 |
| CV% | | 9.07 | 8.90 |
| LSD | | 6.88** | ** |

### Discussion on implications for breeding programs

This study's findings offer critical insights for breeding programs targeting CLS resistance. The identification of moderately resistant genotypes, such as Rampur Mas, BLG 0068-2, and Shekhar, highlights promising candidates for crossbreeding to enhance both yield and disease resistance. The strong correlation between AUDPC and yield also suggests that selecting genotypes with low AUDPC values can directly mitigate yield losses. Future research should consider exploring the genetic basis of resistance mechanisms within these genotypes, as recent studies suggest that integrating molecular markers for resistance traits could accelerate breeding efforts aimed at developing CLS-resistant blackgram varieties suitable for subtropical climates.

## Conclusion

Cercospora leaf spot (CLS), caused by *Cercospora canescens*, poses a significant challenge to blackgram (*Vigna mungo*) production in Nepal, leading to substantial yield reductions in susceptible genotypes. This study screened twelve blackgram genotypes for CLS resistance under field conditions at Tulsipur, Dang. Results showed that none of the genotypes were completely resistant to CLS; however, based on the area under the disease progress curve (AUDPC), ten genotypes—including Rampur Mas and BLG 0068-2—demonstrated moderate resistance, suggesting partial tolerance to disease progression. In contrast, BLG 0066-1-1 and BLG 0035-1 were categorized as moderately susceptible, with higher AUDPC values indicating greater vulnerability to CLS. These moderately resistant genotypes provide valuable genetic resources for breeding programs, supporting the development of blackgram varieties with improved yield and resilience against CLS. The findings underscore the need for continued selection and breeding to enhance resistance, as such efforts are essential to sustainable blackgram cultivation in CLS-prone regions.

## DECLARATIONS

### Authors contribution

Conceptualization: S.S., S.B., S.O. and A.N.; Methodology: S.S., S.B., S.O.; Software and validation: S.S., S.B. and A.N.; Data curation: S.S, and A.N.; Writing—original draft preparation: A.N., S.S.; Writing—review and editing: S.S, A.N., K.U., I.B., K.R.B.; All authors have read and agreed to the published version of the manuscript.

**Conflicts of interest:** The authors declare no conflict of interest.

**Ethics approval:** This study did not involve any animal or human participant and thus ethical approval was not applicable.

**Consent for publication:** All co-authors gave their consent to publish this paper in AAES.

**Data availability:** The data that support the findings of this study are available on request from the corresponding author.

**Supplementary data:** Not Available

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