

# Original Research Article

## Inheritance of Northern Corn Leaf Blight Resistance in Advanced Generations of Maize (*Zea mays* L.)

### Abstract

Developing genetically resistant maize varieties to mitigate yield losses would reduce reliance on chemical control measures. This study underlines the substantial genetic variability and heritability of NCLB resistance within a recombinant inbred line (RIL) population, highlighting the genetic basis of the trait. ANOVA revealed highly significant genetic variability among families at the 1% level, confirming the presence of sufficient variability for effective selection. The progression from F<sub>5</sub> to F<sub>6</sub> generations demonstrated the effectiveness of selection, as seen from reduced AUDPC scores in the F<sub>6</sub> generation. A genetic gain of -7.74 and a heritability estimate of 48% further validated the success of selection based on lower AUDPC scores. The regression analysis supported by the significant positive correlation ( $r = 0.65$ ) revealed a significant association between F<sub>5</sub> selections and the F<sub>6</sub> AUDPC scores, as seen from an R<sup>2</sup> value of 0.42 and a moderate predictive slope of 0.52. These associations suggest that while selection is effective in predicting advanced-generation performance, the residual variability points to the need for incorporating additional genetic or environmental factors to improve predictive accuracy. These findings emphasize the predominance of genetic factors in NCLB resistance and the stability of resistance traits across the two generations, offering insights into the genetic control of the trait. By providing a solid foundation for breeding efforts targeting durable resistance, this study contributes to the development of maize varieties with enhanced NCLB resistance and supports sustainable agricultural practices.

**Keywords:** AUDPC, Heritability, Maize, NCLB, Selection

### INTRODUCTION

Resistance breeding is a critical area of research in crop improvement, particularly for economically significant crops like maize. Maize, the world's most grown staple crop occupies about 193.7 million hectares worldwide, with an average productivity of 5.75 tons per hectare [1]. An extremely versatile crop grown across different ecological zones, maize however is vulnerable to a host of foliar diseases which can severely impair yields and cause economic losses. Northern Corn Leaf Blight (NCLB), caused by *Exserohilum turcicum*, is one such globally prevalent disease of maize [2].

In warm and humid conditions that promote the disease, spindle-shaped lesions form on the leaves. These lesions spread rapidly in susceptible hosts, reducing their photosynthetic capacity and ultimately affecting the yield [3, 4]. These challenges necessitate sustainable management strategies, with resistance breeding emerging as a primary solution since it represents a long-term and sustainable approach.

Breeding disease-resistant genotypes offers several advantages, including reduced reliance on fungicides, increased productivity, and improved environmental sustainability [5]. Moreover, understanding the genetic basis of resistance traits accelerates the development of improved varieties, strengthens food security, and promotes ecosystem stability. Advances in molecular tools, such as marker-assisted selection (MAS), have further enabled researchers to identify genetic markers linked to resistance. By integrating these markers into breeding programs, researchers can develop resilient maize varieties [6] that empower farmers to manage disease pressures effectively and maintain stable crop production.

To complement molecular approaches, robust phenotyping is essential for accurately assessing disease resistance. One of the widely used phenotyping methods is the Area Under the Disease Progress Curve (AUDPC) - a quantitative measure that estimates the intensity of disease spread over time [7]. This method accounts for the dynamic nature of disease progression allowing researchers to compare different

genotypes. AUDPC is especially useful in breeding programs, where it helps in identifying and selecting disease-resistant varieties. It can be used to screen and evaluate large populations for resistance under controlled or field conditions, leading to identification of genotypes that can withstand disease pressures more effectively.

Building on AUDPC as a tool for identifying resistance in maize populations, this study was formulated to investigate the inheritance of NCLB across two selfing generations of maize breeding families developed from a biparental mating, involving a cross between two contrasting lines for NCLB disease. The primary objective was to analyse the genetic inheritance and follow disease progression in the F<sub>5</sub> and F<sub>6</sub> generations to gain insights into the inheritance of NCLB resistance in this biparental population.

## MATERIALS AND METHODS

### Experimental material

A mapping population was developed using two parental lines: RM16 (resistant) and SS1 (susceptible). The F<sub>1</sub> progeny were selfed to advance the population from the F<sub>2</sub> to the F<sub>6</sub> generation. The advanced generations, specifically the F<sub>5</sub> and F<sub>6</sub>, were evaluated for the inheritance of NCLB resistance. Phenotyping was done for 119 F<sub>5</sub> families and 113 selected F<sub>6</sub> individuals over two growing seasons to assess NCLB disease resistance. Field trials were carried out over two consecutive seasons in Ri-Bhoi District, Meghalaya, India, a natural hotspot for NCLB occurrence. Routine intercultural practices were performed throughout the growing seasons to maintain an agronomically healthy standing crop.

### Phenotyping based on AUDPC scores

Phenotyping was based on calculation of the area under the disease progress curve (AUDPC) scores. At the beginning of the growing season, the causal organism was isolated and cultured on potato dextrose agar medium. Inoculum was then prepared and applied by spraying on 25-30 days old seedlings. Once disease symptoms appeared, the progression of the disease was recorded at 3 days interval for a period of 15 days. From this disease progression data, area under disease progress curve (AUDPC) was calculated [8].

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

where, n = total number of observations,  $y_i$  = disease area at the  $i^{\text{th}}$  observation and  $t_i$  = time at the  $i^{\text{th}}$  observation. The recorded AUDPC scores were analysed using basic statistics, including the calculation of the mean, variance, mode, and median, in order to understand the nature of the phenotypic data.

### Statistical analysis

An ANOVA was done to determine if differences for response to NCLB across the families and generations are statistically significant. Frequency distribution analysis and response to selection which included studies on selection differential and genetic gain to ultimately calculate the realized heritability of disease progression was also done.

Here, selection differential  $S = i\sigma_p$

where  $i$  = intensity of selection (10%) and  $\sigma_p$  is the phenotypic standard deviation

Genetic gain (R) was calculated as the difference between mean values of the F<sub>6</sub> generation and the original F<sub>5</sub> generation.

Realized heritability  $h^2$  was calculated as  $h^2 = R/i\sigma_p$

Pearson's correlation and regression analysis to determine the goodness of fit were also performed to understand the nature of association between F<sub>5</sub> families and the selections advanced to the F<sub>6</sub> generation.

## RESULTS

### Analysis of variance (ANOVA)

ANOVA (Table 1) indicated highly significant differences among the families at 1 % level of significance, implying the presence of genetic variability within the RIL population for response to NCLB disease incidence. This variability is important for identifying lines with enhanced resistance to the disease. Conversely, the marginally significant difference observed between generations suggests that the disease pressure experienced by both generations was largely comparable. This finding implies that the heritability of disease response is predominantly governed by genetic factors, with minimal influence from environmental variation. All in all, these results reinforce the stability of the trait across generations, highlighting the role of genetic control over NCLB resistance.

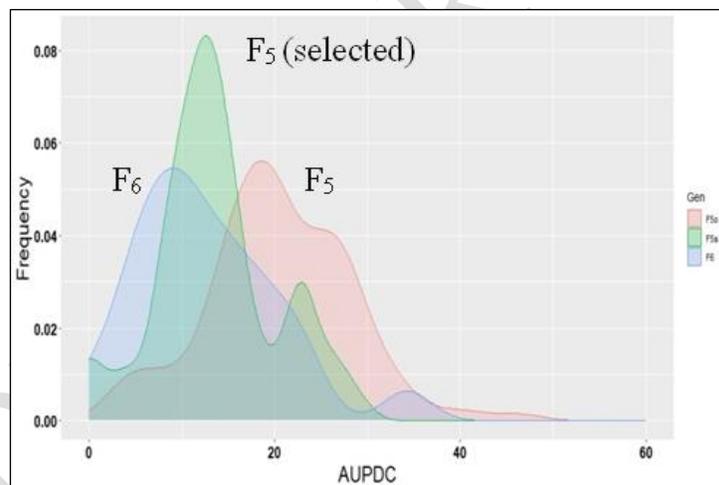
**Table 1. ANOVA of AUDPC Scores for Disease Progression in F<sub>5</sub> and F<sub>6</sub> Generations**

Source of Variation	SS	df	MS	F	P-value	F critical
Genotype (Families)	7964.55	112	71.11**	4.47	0.00**	1.37
Generation	65.70	1	65.70	4.13	0.05	3.93
Error	1782.74	112	15.92			
Total	9812.99	225				

\* $p < 0.05$ ; \*\* $p < 0.01$

### Frequency distribution and response to selection

Based on the frequency distribution studies of the F<sub>5</sub> families, F<sub>5</sub> selections and the F<sub>6</sub> generation, the mean AUDPC score decreased from F<sub>5</sub> (21.03) to F<sub>6</sub> (13.29) as shown in Figure 1. This reflects effective selection, with reduced disease severity in subsequent generations. The median and mode also decreased across generations, indicating increase in the F<sub>6</sub> population's disease resistance.

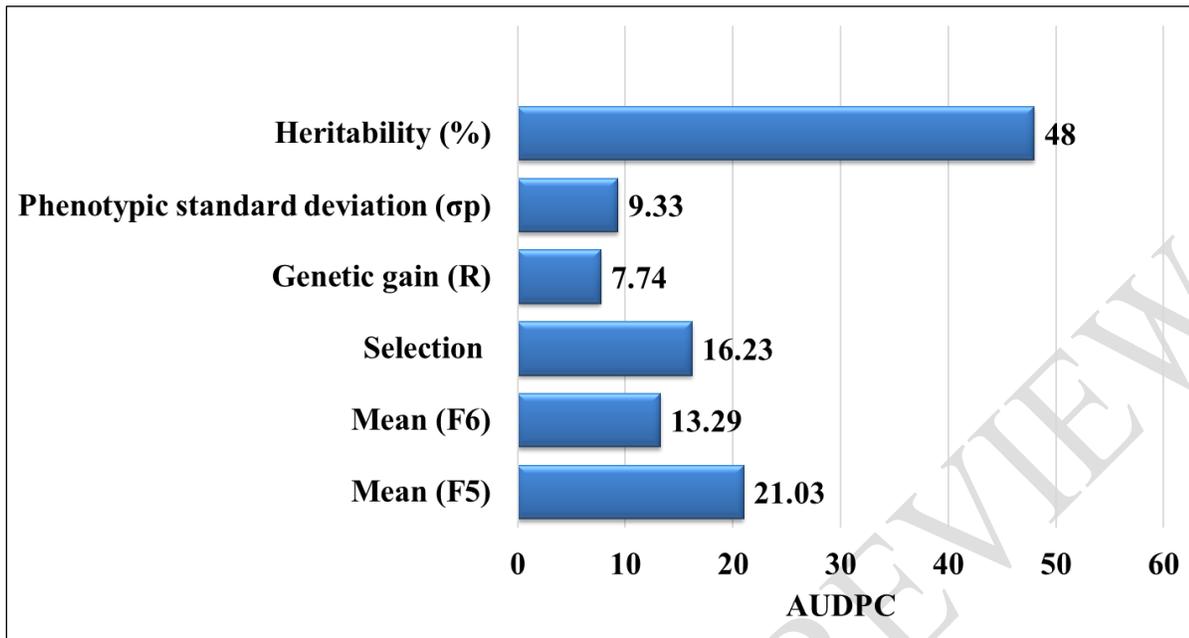


**Figure 1. Frequency distribution graph the F<sub>5</sub> families, selected F<sub>5</sub> RILs and F<sub>6</sub> RILs**

The variability was lower in the F<sub>6</sub> generation, likely due to selection pressure narrowing the range of phenotypes. While the F<sub>5</sub> generation recorded a slight positive kurtosis (0.78), the F<sub>6</sub> generation with a kurtosis of 1, recorded a sharper peak. The right-skewed distribution in the F<sub>6</sub> generation implied that most individuals were concentrated around lower AUDPC scores. The range of AUDPC scores decreased from F<sub>5</sub> (40.94) to F<sub>6</sub> (31.57), further confirming reduced variability. The minimum AUDPC scores decreased from 5.49 in F<sub>5</sub> to 2.75 in F<sub>6</sub>, showing that selection effectively retained the individuals with better resistance.

A selection differential of 16.23 and a genetic gain of -7.74 were achieved for a selection intensity of 10%. The overall phenotypic standard deviation in the F<sub>5</sub> generation was 9.33. Identifying lines with lower

AUDPC scores is desirable, as it signifies higher resistance to NCLB occurrence. The heritability was calculated to be 48% (Figure 2).



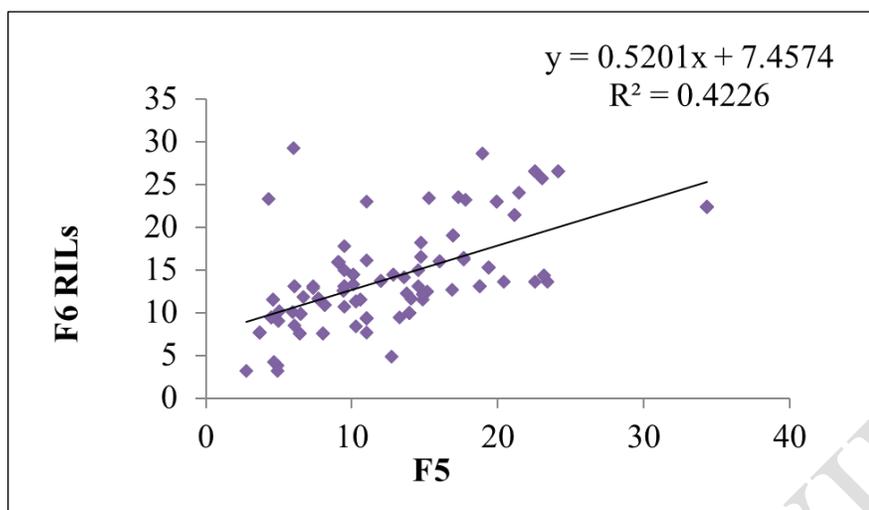
**Figure 2. Response to selection for NCLB disease progress (AUDPC) in the F<sub>5</sub> and F<sub>6</sub> selfing generation**

#### Association studies

Pearson’s correlation studies revealed a highly significant ‘r’ value of 0.65 between F<sub>5</sub> and F<sub>6</sub> generations for AUDPC scores indicating a strong positive relationship between the two generations. For the scatterplot, a positive slope of 0.52, combined with the significant F-statistic and *p*-value (Table 2), confirms that F<sub>5</sub> values have a significant, moderate predictive effect on the F<sub>6</sub> RILs. The R<sup>2</sup> value and the residual variability indicated that while the model captures a meaningful relationship, additional factors not included in the regression (e.g., environmental influences, epistasis, or other genetic effects) account for a considerable portion of the variation in F<sub>6</sub> as well. This analysis highlights the partial predictability of traits in advanced generations (F<sub>6</sub>) based on earlier selection in F<sub>5</sub>, demonstrating the impact of selection while pointing to areas where model refinement or additional variables could improve predictive accuracy (Figure 3).

**Table 2. Regression ANOVA for F<sub>5</sub> and F<sub>6</sub> generations**

Source	df	SS	MS	F	Significance F
Regression	1	2511.22	2511.22	81.23	0.00
Residual	111	3431.67	30.91		
Total	112	5942.89			



**Figure 3. A regression graph depicting the strong association between F<sub>5</sub> families and F<sub>6</sub> selections**

## DISCUSSION

This study comprehensively evaluated genetic variability, heritability, and response to selection for NCLB resistance in maize, focusing on F<sub>5</sub> and F<sub>6</sub> generations of a biparental population. The results provide significant insights into the genetic control of resistance, demonstrating the effectiveness of phenotypic selection on the basis of AUDPC scores to improve resistance breeding strategies. The highly significant genetic variability among recombinant inbred lines (RILs) at the family level, as revealed by ANOVA, highlights the diversity present within the population for resistance to Northern Corn Leaf Blight (NCLB). This variability is a prerequisite for identifying and advancing superior genotypes with enhanced resistance [9, 10]. Additionally, the comparable performance across generations (marginally significant differences) highlights the role of genetic factors in determining resistance, reinforcing the heritability and stability of the trait across different conditions.

The frequency distribution analysis revealed a clear shift in population characteristics due to selection pressure, as seen in the reduction of AUDPC scores from F<sub>5</sub> to F<sub>6</sub> generations. The narrowing variability and sharper kurtosis in F<sub>6</sub> indicated that selection was effective in concentrating resistance traits while screening out the less favorable phenotypes. The right-skewed distribution in F<sub>6</sub>, along with the reduction in range and minimum AUDPC scores, confirmed that selection effectively identified and retained individuals with higher resistance. Notably, the achieved genetic gain combined with a heritability estimate of 48%, highlight the substantial progress made in improving resistance. These results are particularly significant, given that NCLB resistance is influenced by both vertical (monogenic) and horizontal (polygenic) resistance mechanisms [5, 7, 11]. The regression and correlation analyses further emphasized the partial predictability of F<sub>6</sub> generation traits based on F<sub>5</sub> values, as seen from the moderate positive slope, high correlation coefficient ( $r = 0.65$ ), and significant regression statistics. While these results validate the effectiveness of phenotypic selection, the residual variability needs to be addressed in future studies through advanced genomic approaches, such as SNP-based QTL mapping, GWAS (Genome-Wide Association Studies), and genomic selection. These studies would help refine predictive models and enhance the precision of selection efforts [12, 13]. These tools can also help unravel the complex genetic architecture of NCLB resistance, identifying key loci that contribute to disease resistance [14].

To further strengthen the breeding program, multi-environment trials across diverse agro-climatic zones are up next. Such trials would ensure the adaptability and stability of resistant lines [10], particularly in regions with varying disease pressure, soil conditions, and climatic factors. This is especially critical along the foothills of North east India, which relies heavily on organic farming and where maize is an important crop for economic stability [15]. Developing NCLB-resistant varieties for this region supports the goals of sustainable agriculture by reducing yield losses, minimizing fungicide dependency, and promoting

environmentally friendly farming practices.

## CONCLUSION

Overall, by focusing on genetic resistance, the findings contribute to the development of resilient maize varieties. These outcomes have broader implications for maize breeding in other regions with similar environmental conditions and disease pressures. The work done reinforces the potential of genetic improvement to transform maize into a more reliable crop for diverse agricultural systems, particularly in regions like the NEHR, where it serves as a vital component of rural livelihoods and poultry feed production systems.

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