Research Article

Reaction and Diversity Analysis to Identify Novel Genetic Resources for *Exserohilum turcicum* Resistance in Maize

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Abstract | The magnitude and nature of the genetic divergence determine the genotypes for a maize breeding program. One hundred one inbred lines of maize were evaluated using Rampur Hybrid 10 as resistant check in a rod row design from October 2020 to March 2021 at the National Maize Research Program's research farm in Rampur, Chitwan. The goal includes identifying correlations between genotypes, estimating the proportionate contribution of various disease characteristics, and selecting eligible parent lines for hybridization purposes utilizing principal component analysis and Ward's clustering. Correlation assessment revealed a negative relationship between disease parameters and grain yield. According to the principal component analysis, three of the nine main components were significant (eigenvalues > 1) and accounted for 44.60%, 15.30%, and 13.60%, respectively of the total variance. PC1 comprised parameters including percent disease index (PDI), area under the disease progress curve (AUDPC), disease incidence percent (DI%), and infected leaves per plant (IL/P) that were mostly connected to quantitative resistance; PC2 was related to lesion number and lesion breadth; PC3 was mostly concerned with grain yield, while PC4 with sporulation (spores/ml). Inbred lines were divided into three groups using cluster analysis, with 27 inbred lines being placed in cluster II. The mean values of the disease parameter were found to be the lowest in Cluster II, while the grain yield was the greatest. Cluster analysis showed the highest inter-cluster distance between cluster II and cluster III (4.19) and the lowest between cluster I and cluster III (2.24). Clusters I and III have the lowest inter-cluster distance, indicating that their genotypes are quite similar. By utilizing heterosis in segregating, choosing parents from clusters II and III would be successful in the hybridization program to develop improved NLB-resistant hybrids.

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Introduction

The global challenges for maize production include biotic and abiotic stresses, insufficient inputs, poor soil fertility, and low mechanized operations (Prasanna, 2016). In Nepal, poor crop management and diseases are the main factors causing the quantity and quality of maize to decline. Maize is prone to many

fungal pathogens, few bacterial species, and viruses. Seventy-five fungal and only 3 bacterial pathogens have been recorded in Nepal (Subedi, 2015). Southern leaf blight (*Bipolaris maydis* (Nisikado) Shoemaker), northern leaf blight (*Exserohilum turcicum* (Pass.) Leonard and Suggs), gray leaf spot (*Cercospora zeae-maydis* Tehon and Daniels), banded leaf and sheath blight (*Rhizoctonia solani* Kuhn*)* and ear rot are the major fungal diseases prevalent in Nepal (Subedi, 2015). Northern leaf blight (NLB) is a devastating foliar disease of humid and colder areas that needs to be managed effectively. Significant losses were recorded from the disease in Nijgadh, Bara during the winter season of 2012 (NMRP, 2013). Yield losses were reported up to 43.13% at Kakani on local maize, 22% and 33% at Khumaltar on Manakamana-1 and Khumal yellow variety, respectively, and 42.7% at Pakhribas on Hetauda composite under artificially epiphytotic conditions (Rijal *et al*., 2014).

Prevailing maize improvement and disease control strategies include utilization of host resistance, chemical management (Payak and Sharma, 1985), biological and botanical management, pathogen elimination by application of good cultural practices that include farm hygiene, intercropping, residue management, crop rotation and biotechnological approaches (Mueller *et al*., 2020). Although the use of fungicides is not always economical for maize (Mallowa *et al*., 2015), it may benefit large-scale growers, seed producers, commercial and high-value material producers like sweet corn, popcorn, etc. It has the greatest likelihood of economic return if fungicides are applied on susceptible cultivars before the tasseling (VT) or silking (R1) stage at the prudent rate with appropriate spraying strategies (Carpane *et al*., 2020). Side effects and lingering impacts on the environment and public health will become a rising issue due to the development of resistant strains of the pathogen if fungicides are not applied wisely (Barad *et al*., 2019). Cultural and chemical management is not always practical and feasible, and no single approach can successfully manage the epidemic of the disease. Therefore, the principal and most reliable technique for managing northern leaf blight (NLB) of maize is to adopt resistant cultivars, which lowers production costs, decreases management efforts, and reduces environmental concerns (Ribeiro *et al*., 2016; Vieira *et al*., 2009). Even the best way to combat this disease is the application of integrated tactics that include the adoption of resistant varieties, debris, and stubble management, and the use of fungicides only when required.

Genetics of resistance is determined both qualitatively and quantitatively in maize genotypes. Qualitative/ vertical resistance is governed by single or monogenic genes and race-specific *Ht* genes. It is the highest level of resistance conferred by many qualitative genes i.e., *Ht* gene, which may be dominant or partly dominant. Dominant genes (*Ht1, Ht2*, *Ht3*, *HtN*, *HtNB*, *HtP*, *HtM*) and two recessive genes (one described by Carson (1995) and the other is *rt*)) provide resistance to various races of *Exserohilum turcicum*. *Ht1* gene expresses chlorotic lesions with minimum sporulation (Hooker, 1963) whereas, *Ht2 and Ht3* gene expresses a chlorotic lesion with slightly more necrotic lesions than that of *Ht1* (Hooker, 1977, 1981). Similarly, *HtN (*or *Htn1)* gene expresses delayed symptoms until anthesis and *HtNB* gene expresses non-lesion resistance before heading (Wang *et al*., 2012). On the other hand, *HtP* gene expresses full resistance to chlorotic lesion (Ogliari *et al*., 2005) however; *HtM* gene expresses full resistance. The recessive gene mentioned by Carson (1995) expresses a chlorotic lesion of 1 cm diameter and *rt* recessive gene expresses a chlorotic lesion to full resistance (Ogliari *et al*., 2007). Different physiological races of *Exserohilum turcicum* have been reported such as 0, 1, 2, 3, 12, 13, 23, N, 1N, 2N, 3N, 12N, 23N, and 123N. These physiological races are identified based on the phenotypic reaction after inoculation in maize lines (Dong *et al*., 2008; Hooda *et al*., 2017; Turgay *et al*., 2020). Race 0 can infect only susceptible varieties, but Race 1 can infect cultivars with the *Ht1* gene due to the conversion of the avirulence gene into virulence. Till now, Race 123N can infect all cultivars with corresponding *Ht* genes (Galiano-Carneiro and Miedaner, 2017). The *Ht1, Ht2, Ht3,* and *Htn1* genes, which have been backcrossed into common inbred lines, have received the most research attention (Ferguson and Carson, 2007). As the pathogen mutates from avirulence to virulence, the *Ht* genes may become ineffective, or the resistance may be "broken" (Vale *et al*., 2001). The boom-Bust period is likely to occur due to the possible emergence of new races, so qualitative resistance is not believed to be durable and sustainable.

Quantitative/ horizontal resistance is governed by multiple genes (polygenic), non-race specific, and has a small impact on disease resistance. It is primarily expressed as a reduced number and size of lesions,

an increase in the latent period with the decreased amount of sporulation compared to the susceptible genotype (Kumar *et al*., 2011). The most frequent parameters for the assessment of maize genotypes for NLB resistance are disease severity, disease incidence, lesion size, and area under the disease progress curve (Abera *et al*., 2016). Due to the vulnerability of single-gene resistance to the formation of new races, partial resistance is considered more lasting. A combination of monogenic *Ht* resistance with partial resistance allows for additive or complementary effects that might improve the total resistance level (Lipps *et al*., 1997). Already-known characters with high genetic stability, vigor, uniformity, and reproducibility make inbred lines an important resource for research activities (Mubeen *et al*., 2017). Thus, the identification of disease resistance inbred lines could be one of the most important components of an integrated disease management strategy to combat this devastating disease. Knowledge of disease reaction and identification of resistant inbred lines is a great need for the maize improvement program. There is a need to develop, identify, and utilize germplasm with northern leaf blight resistance for the mitigation of the potential loss from disease.

Some of the major works have been already performed on the evaluation of genotypes, which was reviewed in the paper by Subedi (2015). A higher frequency of sexual reproduction of the fungi may increase the risk of generating new races, so identification and evaluation of new sources of resistance to northern leaf blight is the most. Hence, a total of 101 inbred lines were screened under artificial epiphytotic conditions. The goal of the current study was to examine the genetic diversity and discover sources of resistance that may be used in the future to create elite resistant varieties, assess a useful gene pool, and map resistance genes.

Materials and Methods

Meteorological conditions in the experiment site

The experiment was conducted at the National Maize Research Program (NMRP) research farm in Rampur, Nepal. Rampur is 228 meters above sea level and located at 27° 40' 50" North, 84° 19' 3" East. It has a humid and subtropical climate, with mild winters (2-30°C) and scorching summers (up to 43°C). The annual average rainfall exceeds 1500 mm, with a distinct monsoon season (>75% of the total annual rainfall) lasting from mid-June to mid-September (NARC, 2022). The soil was sandy loam, and the meteorological conditions over the whole research period are shown in Figure 1. During our field trial in Rampur, Chitwan, no precipitation was detected.

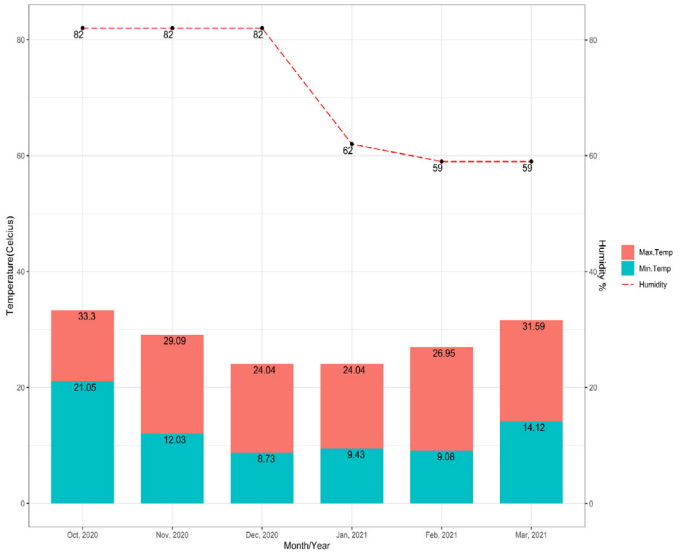

Figure 1: *Meteorological data collected at Rampur, Chitwan during the research period.*
Source: *National Maize Research Program (NRMP), Rampur, Chitwan.*

Single spore isolation and maintenance of pure culture

During the winter of 2020, maize leaves with characteristic northern leaf blight lesions were collected from the research site. Infected leaves were air-dried, placed in a paper bag, and refrigerated at 4°C for further pathogen isolation. Fungus was isolated from diseased leaf samples using the technique reported by Shekhar and Kumar (2012). The sterilized knife was used to cut a small amount of diseased leaf tissue with some nearby healthy tissue measuring approximately 5 mm × 5 mm. Surface sterilization was performed to destroy any undesired surface pathogens by immersing the leaf parts in 75% ethanol for 30 seconds and 1% sodium hypochlorite solution for 1 minute before washing three times with distilled water. To absorb moisture, the leaf sections were wiped using sterile filter paper. The leaf fragments were put in a sterile petri plate with three layers of wet blotting paper to create a moist chamber for fungal sporulation at 25±2°C in a BOD (Bio-oxygen demand) incubator for 24 hours.

A single spore on the surface of the lesion was picked up with the help of fine flattened needles under a stereomicroscope and placed on water agar (20 g agar/l of distilled water) aseptically. After the germination

of the spore in 24 hours, a single spore was again transferred from water agar to separate culture tubes of potato dextrose agar (PDA) (200 g potato infusion, 20 gm dextrose, 20 gm agar for 1 lt) slants with the help of stereomicroscope and inoculating needle under laminar flow chamber aseptically. Streptomycin sulfate (50 ppm) was added to the PDA to limit bacterial development. To get the pure monoconidial isolates, the tubes were cultured in a BOD incubator at 25±2°C for 12 days. PDA slants were kept in the refrigerator at 4°C for short-term storage as a pure stock culture for future study. This isolation approach is also included in Sun *et al.* (2020).

Genotypes, experimental sites and design

NARC provided 101 maize inbred lines, which were employed in the study under artificial epiphytotic conditions as given in Table 1.

The field trial was carried out during the 2020 winter season. Each genotype was planted in a two-row plot of 4 m length with 75cm ×20 cm spacing using a maize jab planter. Rampur Hybrid-10 and Rampur Composite were employed as resistant and standard checks, respectively. The consistent number of plants was maintained by eliminating the surplus plants 15 days after sowing. The fertilizer dose used was 6 t/ha FYM and 120:60:40 NPK (urea, di-ammonium phosphate, and muriate of potash) where 50% N was administered at basal and the rest 50% at knee high (35 DAS) and tasseling stage (80 DAS). Weeding and hoeing were done before tasseling at the knee-high stage. Irrigation was performed at key times, such as knee-high, tasseling, and silking. Emamectin benzoate (0.4 g/L) and Spinosad (0.3 ml/L) were sprayed alternatively in the evening for three days at a 10-day interval to control autumn armyworms in the field.

Artificial inoculation

The initial spray of the inoculum was done using a hand atomizer at a concentration of 2.25 $\times 10^4$ on the 35th day after sowing during the twilight hour. The spray was often administered to the whorl of the plants (whorl placement method), where it was maintained for a longer time enough to facilitate spore germination. The second spray was applied 15 days following the first vaccination. For consistent dissemination over the leaves, Allvit, a surfactant that works as a spreading and wetting agent, was combined at 1 ml/lt. Spraying water caused high humidity (>90% relative humidity) and leaf wetness for the next two days to encourage disease growth (Abera *et al.*, 2016).

Table 1: *List of maize genotypes used for screening against northern leaf blight under artificial epiphytotic conditions during 2020/21 in Rampur, Chitwan.*

E. N	Genotypes	E.N.	Genotypes	E.N.	Genotypes
1	RL_100	35	RL_265	69	RML_57
2	RL_101	36	RL_270	70	RML_58
3	RL_105	37	RL_271	71	RML_62
4	RL_111	38	RL_272	72	RML_65
5	RL_133	39	RL_279	73	RML_68_1
6	RL_150	40	RL_280	74	RML_68_2
7	RL_153	41	RL_281_1	75	RML_76
8	RL_165	42	RL_281_2	76	RML_83
9	RL_173	43	RL_283	77	RML_84
10	RL_13	44	RL_286	78	RML_85
11	RL_180	45	RL_288	79	RML_86
12	RL_202	46	RL_290	80	RML_87
13	RL_21	47	RL_291	81	RML_88
14	RL_213	48	RL_297	82	RML_89
15	RL_215	49	RL_142_2	83	RML_93
16	RL_217	50	RL_30_3	84	RML_96
17	RL_221	51	RL_35_1	85	Pop_corn_2
18	RL_293	52	RL_84	86	Pop_corn_Gorkha_3
19	RL_229	53	RML_107	87	Pop_corn_ madhyapahad
20	RL_232	54	RML_114	88	Pop_duplicaiton
21	RL_99	55	RML_115	89	Pop_corn_Y+W(Y)
22	RL_235	56	RL_269	90	Australian_1_ sanodana
23	RL_236	57	RML_138	91	Madhyapahad_ ratokande
24	RL_238	58	RML_142	92	Australian_ thulodana(W)
25	RL_239	59	RML_144	93	Pop_corn_ budhokande2
26	RL_240	60	RML_146	94	ID_8002(w)
27	RL_241	61	RML_147	95	ID_7147(w)
28	RL_242	62	RML_149	96	ID_8007®
29	RL_243	63	RML_150	97	ID_8004Y®
30	RL_244	64	RML_170	98	ID_7964(Y)
31	RL_246	65	RML_188	99	ID_8007YR®
32	RL_248	66	RML_191	100	Rampur Composite
33	RL_249	67	RML_2	101	Rampur Hybrid 10
34	RL_251	68	RML_4		

Table 2: *Disease rating scale of northern leaf blight of maize.*

Rating scale	Degree of infection
1	Plants with one or two to few scattered lesions on lower leaves (Resistant)
2	Moderate number of lesions on leaves, affecting <25% of the leaf area (Moderately Resistant)
3	Abundant lesions on lower leaves and few on other leaves affecting 26-50% of leaf area (Moderately Susceptible)
4	Lesions abundant on lower and mid leaves, extending to upper leaves affecting 51-75% of leaf area (Susceptible)
5	Lesions abundant on almost all leaves, plants prematurely dried or killed with 76-100% of the leaf area affected (Highly Susceptible)

Disease assessment

Estimation of disease reaction and scoring: Ten plants were randomly selected and labeled at each plot before inoculation. Such plants were utilized for disease evaluation at 10-day intervals beginning when 2-3 lesions were identified in basal leaves, using a 0-5 grading scale as given by CIMMYT (1985), Singh *et al*. (2004) as shown in Table 2. The initial scoring occurred 60 days after sowing and was repeated six times (i.e., six severity scores).

Estimation of infected leaves, lesion number, and lesion size: The total number of infected leaves from 10 tagged sample plants was tallied across all six scoring days. The total number of lesions was also calculated using two leaves from each sample (one from the ear and one above the ear). To estimate the rate of lesion expansion, a lesion from each plant sample was marked with a red fabric, and its size (length and width in mm) was measured each time with a digital vernier caliper (Abebe *et al*., 2008). The number and size of lesions were assessed on the 70th, 80th, and 90th days following sowing (i.e., on the 2nd, 3rd, and 4th scoring).

Estimation of disease incidence percentage: It refers to the proportion of experimental individuals who have the disease symptoms out of the total number of experimental subjects tested. The incidence of each treatment for northern leaf blight was visually assessed at weekly intervals in all plots beginning with the first symptomatic appearance. For each plot, the number of infected maize plants was counted and expressed as a percentage of all maize plants. The percentage of disease incidence was calculated by using the formula of Wheeler (1969) as:

$$\text{Disease incidence \%} = \frac{\text{No. of infected plants}}{\text{Total no of observed plants}} \text{ X } 100\%$$

Estimation of percent disease index (PDI)/ disease severity percentage/ percentage severity index (PSI): It simply refers to the severity of the disease infection. The disease score was transformed into a severity percentage using Wheeler's (1969) algorithm as follows:

$$\text{Disease severity \%} = \frac{\text{(Sum of all numerical ratings)}}{\text{No. of plants observed X Maximum reading of scale (i. e. 5)}} \text{ X } 100\%$$

Estimation of Area under the disease progress curve (AUDPC): It is a quantitative measurement of the progression of the disease. The AUDPC was calculated using the disease severity data by the formula given by Campbell and Madden (1990).

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

Where "t" is the time of each reading, "y" is the percent disease severity at each reading and "n" is the number of readings.

Estimation of sporulation in lesion

After the final scoring, leaves with identifiable lesions were separated from five different sample plants representing all treatments and put into labeled paper bags. From each leaf, lesions of 1 cm^2 were measured, a set volume of distilled water (1 ml) was added to a petri plate, the lesion was scraped for two minutes with the forceps and needle to release conidia for conidial measurement with three replications. The number of conidia in the suspension was measured using the haemocytometer based on 5 observations per leaf for minimizing the error.

Estimation of grain yield

Grain yield was assessed by using the formula as mentioned by Tandzi and Mutengwa (2020).

$$\text{Grain yield } \left(\frac{\text{ton}}{\text{ha}}\right) = \frac{\text{FEW } \left(\frac{\text{kg}}{\text{plot}}\right) \times (100 - \text{HMC}) \times \text{S} \times 10000}{(100 - \text{DMC}) \times \text{NPA} \times 1000}$$

Here, FEW= Fresh Ear weight, HMC= Moisture content at harvest, DMC= Desired moisture content i.e., 14%, S= Shelling coefficient i.e., 0.8, and NPA= Net plot area in m^2

Statistical data analysis

The experiment was carried out under field conditions using a rod row design with two rows. The experiment's results were entered and saved in Microsoft Excel. Multiple packages were used to do multivariate analysis in R (4.0.2). Using the Facto Mine R and factoextra packages, the Ward's techniques of cluster analysis, boxplot, correlation analysis, principal components analysis, eigenvalues, eigenvectors, and 2D biplots were achieved.

Results and Discussion

Correlation assessment and principal component analysis

The correlation coefficient between different disease parameters and yield clearly represented some aspects of the relationship as shown in Figure 2. All were significant at 0.05% level of significant. The disease parameters like percent disease index (PDI), area under disease progress curve (AUDPC), disease incidence % (DI %), infected leaves per plant (IL/P), lesion length (LL), lesion breadth (LB) and sporulation (spores/ml) were positively correlated with each other while all were negatively correlated to the grain yield (t/ha) except lesion number (LN) which nearly showed no relationship. These positive correlation coefficients between disease components indicated that it has the direct relationship between the components to cause the northern leaf blight of maize. The negative correlation coefficient between disease components and grain yield signifies that an increase in the intensity of disease parameters directly hampers the yield production.

The results of the principal component analysis (PCA) clearly showed that three of the nine principal components were significant (eigenvalues > 1) and accounted for 73.5% of the variance. According to Figures 3 and 4, PC2 and PC3 each accounted for 15.30% and 13.60% of the variation, respectively, with PC1 accounting for the largest variance (44.60%). For future analysis, only these three components with eigenvalues greater than 1 were taken into consideration. The overall variance in the data was better described by principal components with eigenvalues > 1 than by individual quality. The proper

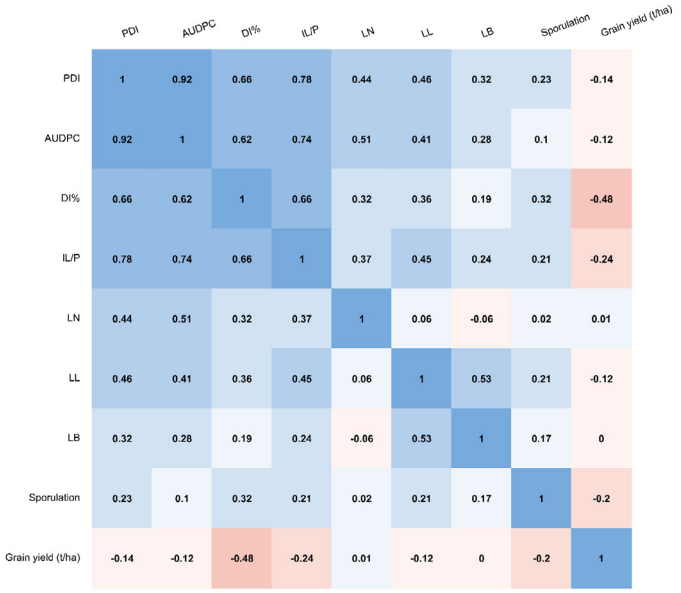

Figure 2: *Correlation coefficient between eight components of disease development and grain yield of 101 maize inbred lines (PDI: Percent disease index, AUDPC: Area under the disease progress curve, DI %: Disease incidence %, IL/P: Infected leaves per plant, LN: Lesion number per leaf, LL: Lesion length in mm, LB: Lesion breadth in mm).*

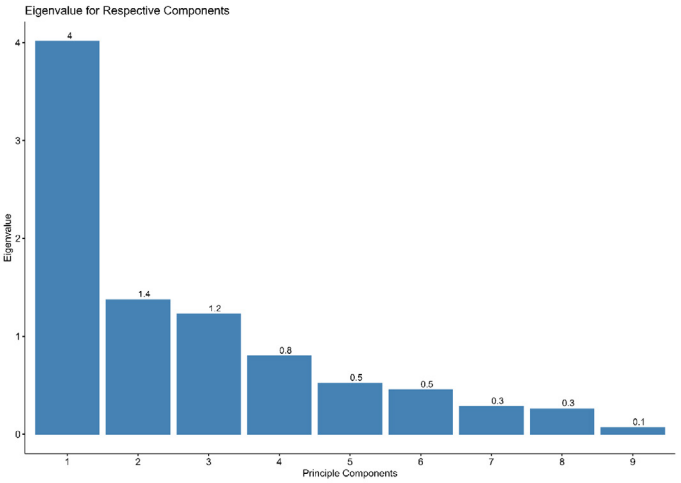

Figure 3: *Eigenvalues of different principal components as shown by principal component analysis of maize inbred lines.*

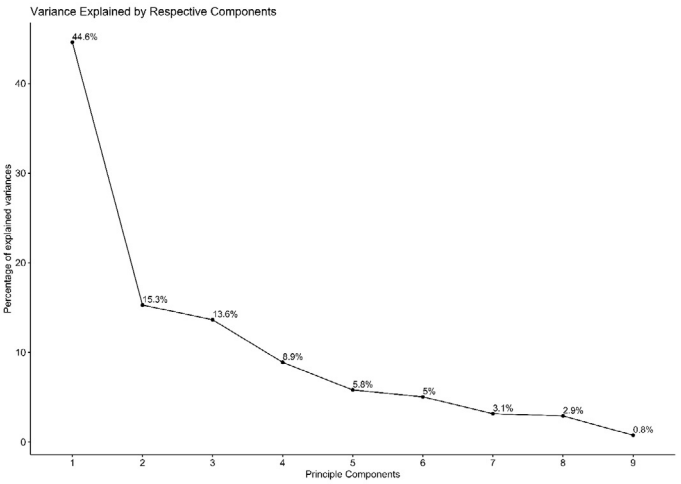

Figure 4: *Each principal component contributes to the total explained variance in the diversity of maize inbred lines based on various disease parameters and grain yield.*

value evaluates the significance and contribution of each component to the overall variance, whereas each coefficient of the proper vector displays the percentage of each original variable's contribution to the major component it is related to. The higher the coefficient increases, regardless of its sign, the more successful the relevant characteristics will be in identifying inbred lines (Dhakal *et al.*, 2020).

Figure 5: *The principal component analysis and component value for 8 different disease parameters and grain yield of 101 maize inbred lines. The blue hue represents the maximum positive impact, while the red color represents the lowest positive or no contribution (PDI: Percent disease index, AUDPC: Area under the disease progress curve, DI %: Disease incidence %, IL/P: Infected leaves per plant, LN: Lesion number per leaf, LL: Lesion length in mm, LB: Lesion breadth in mm).*

As seen in Figure 5, PC1 was generally related with traits related to quantitative resistance, PC2 was related to lesion number and lesion breadth, PC3 was mostly concerned with grain yield, and PC4 with sporulation. In PC1 (44.60%), the variables like PDI (0.84), AUDPC (0.78), infected leaves per plant (0.75), and DI % (0.66) were positively correlated. The second principal component PC2 (15.30%), showed a positive correlation with lesion number (0.42) and lesion breadth (0.38). PC3 (13.6%) showed a highly positive relationship with grain yield (0.6) while PC4 was contributed positively by sporulation (0.56). The magnitude and direction of various traits in the different principal components are represented as a biplot as shown in Figures 6 and 7.

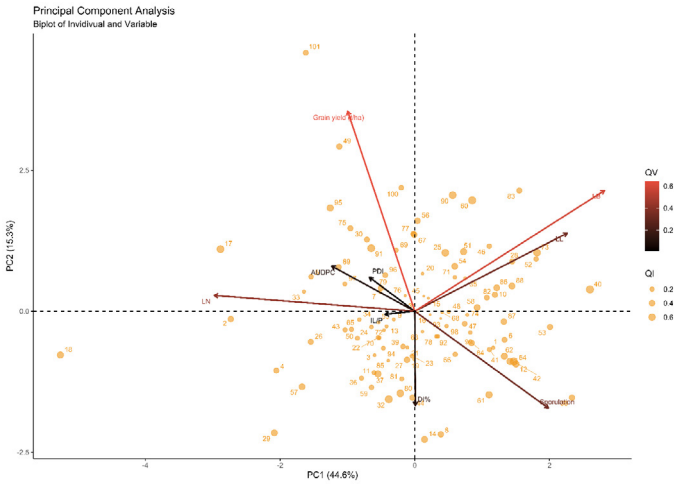

Figure 6: *The biplot of 101 maize inbred lines for PC1 and PC2. The arrows depict the size and direction of the trait's impact in PC1 and PC2. Individual quality of representation (QI) and variable quality of representation (QV) are two different concepts.*

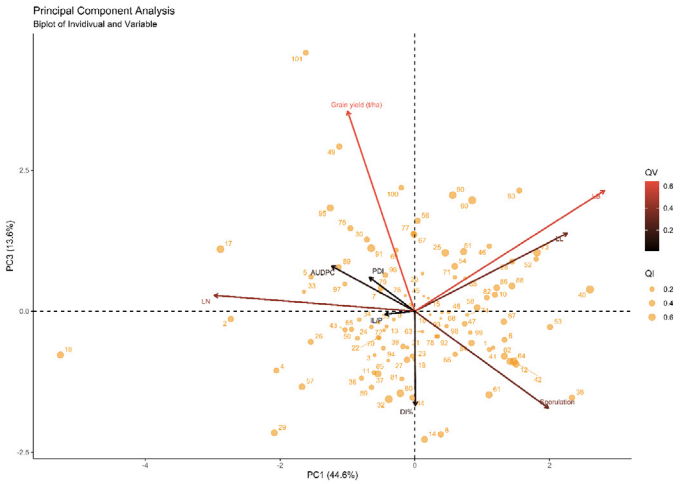

Figure 7: *The biplot of 101 maize inbred lines for PC1 and PC3. The arrows depict the size and direction of the trait's impact in PC1 and PC3. Individual quality of representation (QI) and variable quality of representation (QV) are two different concepts.*

Ward's method and euclidean distance

Grouping of 101 maize inbred lines were done by using Ward's minimum variance clustering method which is very appealing as it minimizes within cluster variance. The maize inbred lines were grouped into 3 clusters as listed in Table 3. The number of clusters was determined by using the gap statistic method as represented in Figure 9. Clustering was done based on various disease parameters contributing to quantitative resistance like percent disease index (PDI), disease incidence % (DI %), AUDPC, infected leaves per plant (IL/P), lesion number per leaf (LN), lesion length (LL), lesion breadth (LB), sporulation (spores/ml)

Table 3: *Distribution of 101 genotypes of maize in 3 different clusters for identification of resistant sources.*

Cluster number	Genotypes serial Numbers	Total number of genotypes
1	1,6,8,10,12,14,19,25,28,30,38,40,42,48,51,53,54,58,61,62,64,67,73,78,84,86,87,88,90,91,96,97,98	33
II	2,3,4,11,21,29,31,33,36,41,44,46,57,59,63,74,76,81,82,83,85,92,93,94, 99,100,101	27
III	5,7,9,13,15,16,17,18,20,22,23,24,26,27,32,34,35,37,39,43,45,47,49,50,52,55,56,60,65,66,68,69,70,71, 72,75,77,79,80,89,95	41

and grain yield (t/ha) shown in Figure 8. The inter-cluster distances among 3 clusters were shown in Table 5 which is used to measure the genetic divergence among the maize inbred lines.

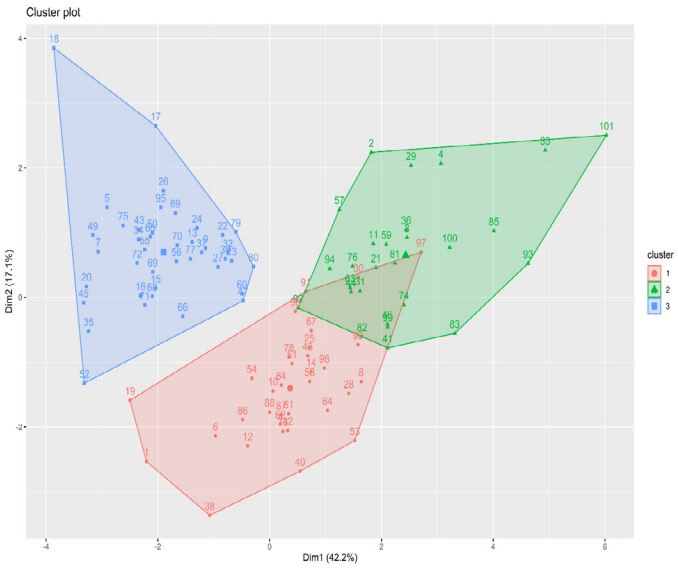

Figure 8: *Hierarchical clustering of 101 genotypes using Ward's method for identification of resistant sources against NLB of maize under field conditions at Rampur, Chitwan during 2020/21.*

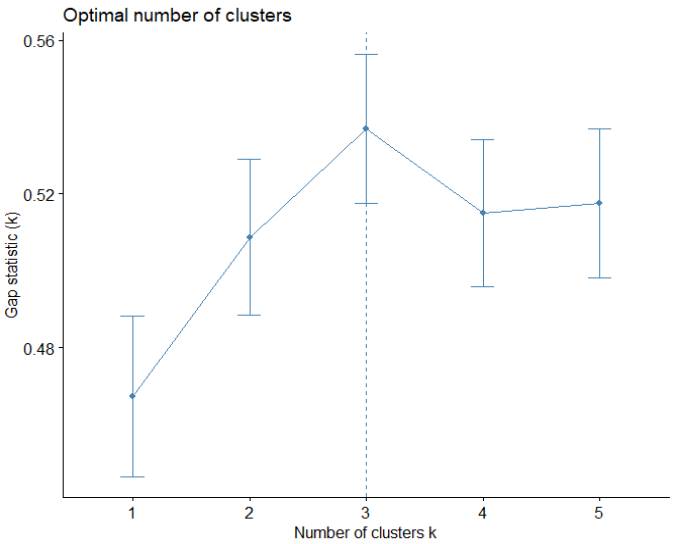

Figure 9: *Gap Statistic method to determine the optimum number of clusters.*

From Table 3, Cluster III was the largest cluster as it comprises 41 inbred lines, while cluster I and cluster II included 33 and 27 inbred lines, respectively. Mean performance of different components in each cluster from Table 4 revealed that the lowest values for PDI (24%), AUDPC (725), DI % (63%), infected leaves per plant (2.3), lesion number per leaf (1.79), lesion length (55 mm), lesion breadth (5.94 mm) and sporulation (0.78×10^4) was found in cluster II. Highest grain yield (1.6 t/ha) was also reported in cluster II. The clustering clearly revealed that the genotypes under cluster II might be promising for further maize breeding programs. The highest inter-cluster distance was found between cluster II and cluster III (4.19) and the lowest was found between cluster I and cluster III (2.24). It revealed that genetic variation exists among 101 inbred lines against northern leaf blight. Similar to this study, Pasha *et al.* (2013) used cluster analysis with Ward's method and Euclidean distance criteria to divide the rice genotypes into three groups based on field traits (infection type, panicle blast severity, panicle and leaf blast disease progress). All resistant varieties were placed in the first group. Sultana *et al.* (2018) revealed that the pathogenic variation exists among 169 isolates from mean-inter cluster distance values. The inter-cluster distance is due to the heterogenous nature of the inbred lines between the clusters. The cluster's inbred lines were closely connected, as shown by the lowest distance (Dhakal *et al.*, 2020).

Table 4: *Mean performance of different components in each cluster.*

Characteristics	Cluster I, N*=33	Cluster II, N= 27	Cluster III, N=41
Percent disease index (PDI)	38	24	52
AUDPC	1195	725	1895
Disease incidence %	86	63	95
Infected leaves per plant	3.64	2.30	5.47
Lesion number per leaf	2.05	1.79	3.32
Lesion length (mm)	79	55	77
Lesion breadth (mm)	7.51	5.94	7.14
Sporulation (spores/ml)	2.22×10^4	0.78×10^4	1.29 x 104
Yield (t/ha)	1.28	1.61	1.09

N represents the number of genotypes in the cluster.*

Table 5: *Mean intercluster distance values of each cluster of 101 maize inbred lines.*

	Cluster 1	Cluster 2	Cluster 3
Cluster 1	0		
Cluster 2	2.82	0	
Cluster 3	2.24	4.19	0

To guarantee the successful and efficient use of hybridization programs, a diversity study of *Exseohilum turcicum* infected maize inbred lines is crucial. The greater the inter-cluster distance range, the more diverse the populations are. Divergence values can be used to determine the cluster type for further selection and to choose the parents to employ in hybridization or breeding operations (Jagadeb and Samal 1991). The genetic diversity between and within clusters increases with increasing intra- and inter-cluster distances. To achieve maximal heterosis in hybridization and to be employed in the crossover program to get a wide range of diversity among the segregated generations, the selection of parents from distant groups was thus predicted (Sharma *et al.*, 2020). Therefore, selecting parents from clusters II and III might be useful for producing long-lasting NLB-resistant cultivars. In contrast, clusters I and III had the smallest inter-cluster distance, indicating that their genotypes were the closest to one another. As a result, selection will not work since their genetic distance was just estimated.

Variability of quantitative traits for aggressiveness

The boxplot in Figure 10 represents the basic statistics of different traits, which contribute to the quantitative resistance of the host. Most important disease parameters like AUDPC (725), PDI (24) and DI % (63) has lowest mean value in the genotypes of Cluster II, whereas the highest mean values (1895, 52 and 95, respectively) were reported in the genotypes of cluster III. Major components of the aggressiveness of the pathogens are sporulation (0.78 x 10^4 spores/ml), lesion length (55 mm), and lesion breadth (5.94 mm) which also has the lowest mean value in the genotypes of cluster II. The mean yield was reported highest (1.61 t/ha) in the genotypes of cluster II while minimum mean yield in cluster III (1.09 t/ha).

In NCLB studies, resistance expression included lesion type, disease score, area under the disease progress curve (AUDPC), yield loss, kernel weight loss, number of lesions per plant, lesion size and number of conidia produced on lesion segments (Zhu *et al.*, 2023). Quantitative trait variation among populations is a fundamental need for adaptation. The pathogen life cycle divides aggression into simple quantitative features. Typically, epidemic rates are used to directly assess aggression (Cumagun and Miedaner, 2003). Aggression is measured by a range of quantitative features that are manifested during the host-pathogen interaction between genotypes of plants and pathogens. These characteristics known as aggressiveness components include lesion size, spore production rate, infection efficiency, and latent time (Sackett and Mundt, 2005). Their capacity for reproduction has an impact on their ability to spread disease. Sporulation is measured by the number of spores produced per infected leaf area (Clifford and Clothier, 1974).

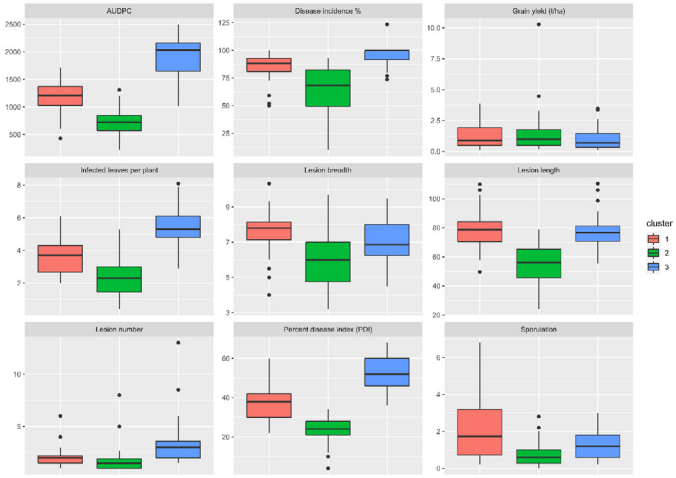

Figure 10: *Box plot of each cluster for various disease parameters and grain yield: mean with standard error value of 101 maize inbred lines.*

Conclusions and Recommendations

The armaments race between host and pathogen is a persistent occurrence in evolution. The goal of gathering, examining, and characterizing unexplored genotypes is to find donors with numerous NLB resistance genes who can be employed right away in breeding operations. The unique inbred lines like RL_101, RL_05, RL_111, RL_180, RL_99, RL_243, RL_246, RL_249, RL_270, RL_281_1, RL_286, RL_290, RML_38, RML_144, RML_150, RML_68_2, RML_83, RML_88, RML_89, RML_93, Popcorn_2, Madhyapahad_ratokande, Popcorn_budhokande_2, ID_8002(w), and ID_8007YR® having resistance to northern leaf blight can be explored for the discovery of novel genes for broadening the gene pool to combat the pathogen competition. It was successfully

established utilizing Ward's clustering and genetic divergence analysis using Euclidean distance. The inbred lines' rich diversity was separated into clusters, and this process revealed the inter-cluster distance between populations and individuals, which may be used to pick individuals from different clusters. It is hard to predict whether or not Ht genes will contribute to more effective long-term management of NCLB, although they may provide some disease protection when quantitative resistance is increased and introgressed into well-known maize lines or when paired with quantitative resistant traits. The knowledge gained from this investigation will aid in the preservation and use of priceless inbred lines of maize. But the research lacks multi location trial and genetic-environment interaction so this might be the future scope of the research.

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Novelty Statement

The research focuses the overall performances of the genotypes of maize from various dimension for the resistance against northern leaf blight.

Author's Contribution

Shishir Sharma: Conduct research process, data generation, data analysis, and draft preparation.
Suk Bahadur Gurung: Data generation, manuscript proofreading.
Ritesh Kumar Yadav: Design research, proofreading, and data analysis.
Bibek Phularan and Laxmi Prasad Joshi: Data collection.

Data availability
The corresponding author is willing to provide the data that back up the study's conclusions upon reasonable request.

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Any opinions, results, conclusions, or recommendations expressed in this publication are solely those of the authors and do not necessarily represent the views of the institutions with which they are associated.

Conflict of interest
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