HISTO-PATHOLOGICAL OBSERVATION OF RESISTANT AND SUSCEPTIBLE MAIZE (*ZEA MAYS* L.) GENOTYPES UNDER *EXSEROHILUM TURCICUM* STRESS

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Abstract. Northern Corn Leaf Blight (NCLB) is the most common fungal disease in the temperate climate zone including Azad Kashmir. It is caused by the fungus *Exserohilum turcicum,* which produces spores that colonizes maize leaf surface. This pathogen damages the leaf structure, causing chlorosis and loss of photosynthetic activity. Compromised maize germplasm demonstrates a variety of NCLB symptoms as well as mechanisms of disease resistance. Planting resistant maize varieties has been demonstrated to be the most effective method of NCLB management. In this report, histochemical studies were conducted to identify the genetic source of maize resistance to *E. turcicum*. Plant susceptibility to NCLB was evaluated on a scale of 0–5. Leaves sample were visually assessed based on symptoms conferring to NCLB stress. Pathogenic assessments explore 3 highly susceptible (HS), 3 highly resistant (HR), 1 resistant (R), 14 moderately resistant (MR) and 9 moderately susceptible (MS) genotypes. The reaction of each tested genotype was again confirmed/evaluated using histochemical observations. Data was recorded for number of infections (NI), fungal colony area (FCA), necrotic area (NA), and the calculated hypersensitivity index (HI). Leaf samples were collected at 10- and 20-days post infection (dpi), allowing comparison of early and full progression of the disease. Results at 10 dpi demonstrated that resistant genotypes had few-to-no infections while demonstrating the highest necrotic area. The largest NA was measured in genotypes Karamat-Bar (3456.66µm), NCEV-1530-11 (726.57µm), and SZP-13200 (625.68µm). Smallest FCAs were observed in Karamat-Bar (50.67µm), NCEV-1530-11 (59.12µm), and SZP-13200 (97.68 µm) at10dpi. Resistance was indicated by HI, which was the highest in Karamat-Bar (93.37%), SZP-13200 (93.09%), and NCEV-1530-11 (90.36%). Histo-chemical screening identified as susceptible genotypes showed larger NA (16µm in Soan-3) and greater FCAs (Soan-3 3943.04µm, Ghuari 2302.73µm, and Kissan 1947.14µm) respectively. These genotypes exhibited the lowest HI (2.6% in Soan-3, 2.51% in Ghuari, and

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2.41% in Kissan). At 20 dpi, NA reduction was observed in resistant genotypes. Decreased disease progression and infection delay is linked to the presence of a resistance gene. Identification of novel resistant maize source and their implementation in future breeding programs could play key role in preventing significant future yield losses.
Keywords: *northern corn leaf blight, susceptibility, necrosis, hypersensitive index, resistance, yield loss*

Introduction

Northern Corn Leaf Blight (NCLB) is an economically significant foliar disease of maize caused by the fungus *Exserohilum turcicum,* a member of the Ascomycete family (Hooda et al., 2017). It is widespread in the temperate climatic region of the world where maize is commonly grown. It is a host-specific pathogen and infects the surface of maize leaves, causing substantial loss in photosynthetic efficacy. 40-70% corn yield losses have been reported in susceptible cultivars at 2-3 weeks post pollination (Haque et al., 2022). In highly susceptible varieties, yield losses can reach as high as 70-100%, causing alarming economic loss (Haque et al., 2022). Maize yield losses correlated with disease severity, resistance level, pathogen interaction and degree of pathogenicity.

NCLB overwinters in plant debris and spreads by wind and rain under suitable conditions (Smith et al., 2004). Conidial germination in *E. turcicum* is bipolar and initiates after 3-6 hours of inoculation, infection proceeding as a germ tube develops from apical cells entering the leaf through stomata (Sørensen et al., 2017). Spreading throughout epidermal tissue, the fungus causes lesion formation and necrosis. Evidence of infection is given by lightening of chloroplasts under staining and finally collapse of this organelle (Fathima et al., 2023). Fungal lesions vary from 6-15cm in length as hyphae grow from xylem cells into the surrounding healthy tissues. Plant cell protoplasm becomes granular during plasmolysis, causing rapid death and severe chlorosis (Hotz et al., 2023).

Certain maize strains demonstrate resistance against NCLB infection. Resistant hybrids demonstrate fewer hyphae pegs in their xylem then in susceptible hybrid leaves (Zhu et al., 2021). The lesions and fungal colony area of resistant cultivars are also smaller compared to the susceptible plants. Resistant cultivar plant cells do not display the rapid decline and mortality of their susceptible counterparts, often avoiding cell infection entirely. In contrast, susceptible cultivars demonstrate deep lesions and bulky air spaces. Infected cells demonstrate initial cell nuclei expansion which then halts (Wise et al., 2019).

Despite of significant research clarifying host response to *E. turcicum* incursion, little has been done using light microscopy, which is essential for the further characterization of resistance mechanism. Histochemical studies are strategic in interpreting host-pathogen interactions in both resistant and susceptible cultivars. Foliar fungicides are reported as an effective management strategy for NCLB (Balint-Kurti, 2019). Fungicide application protects as much as 75% of maize leaf area, minimizing disease impact during the crucial grain filling stage and resulting in significant yield loss reduction (El-Baky and Amara, 2021). To guarantee those emerging seedlings are infection free, application of fungicide before tassel emergence reduces the fungal conidial formation (Rehman et al., 2021). A comprehensive management strategy for NCLB includes cultivating resistant hybrids crop rotation regular monitoring of field disease pressure and weather conditions and application of fungicides before the flowering stage (Mideros et al., 2018).

Genetic studies have determined the key role *Ht* genes have in retarding *E. turcicum* virulence and in reducing sporulation. Cutting-edge techniques in maize horticulture focus on manipulation of resistant genes into maize hybrids through breeding techniques.

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Gene-focused corn cultivation has demonstrated efficacy in disease management and better yield (Reddy et al., 2013). *Turcicum* leaf blight of maize incited by *Exserohilum turcicum*: A review. International Journal of Applied Biology and Pharmaceutical Technology, 5, 54–60. Though resistant maize strains are an effective barrier to disease spread, new *E. turcicum* strains require continued development of mitigation strategies, especially in areas where NCLB is prevalent (Degani et al., 2021). This study was conducted with an aim to identify and characterize the maize genotypes conferring resistance (Ht gene) against *E. turcicum* and their application under local climatic conditions that was hypothesized to be helpful in reduction of yield losses.

Material and methods

The study was conducted in experimental field of the Department of Plant Breeding and Molecular Genetics, University of Poonch Rawalakot, Azad Kashmir in summer 2020. Maize germplasm containing thirty genotypes was cultivated in randomized completely blocked design (RCBD). The seeds beds were prepared and seeds were cultivated in well prepared soil with plant-plant distance of 25cm and row-row distance of 75cm. All required cultural practices, including hoeing and weeding were made. Optimal doses of fertilizers (NPK" (360 kg/ha), Urea (180 kh/ha) were also applied in order to attain best results.

Inoculum preparation

Inoculum was prepared by collecting infected leaves from maize natural field during 2019. The samples were kept in sunlight to become dry and then transfer to oven at 40 °C for 48 hrs. After drying, the samples were grind using mortar and pestle to make inoculum in fine powder form (Ali et al., 2014). The prepare inoculum was preserved for next season experiment.

Artificial inoculation

Inoculum (5 g) was dropped in leaf whorl at 4-6 seedling leaf stages using manual method as prescribed by Ohunakin et al. (2019b).

Germplasm screening

The germplasm was screened conferring various responses/symptoms against NCLB using 0-5 rating scale (*Table 1*).

Sample collection for histochemical observation

Leaf Samples were harvested from field in envelopes showing various susceptibility levels and subsequently labeled (*Table 1*). Samples were then preserved in a freezer at -82ºC and were subjected to the following steps for histochemical analysis.

Fixation of leaf sample and clearing

Leaf samples were fixed using the trypan blue staining method given by Cooksey (2014). Observations were made on 10 and 20 dpi Leaves were collected in replicates; three treatments per observation were recorded. Samples were cut at 2×3 cm^3 and fixed.

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Table 1. *Disease rating scale 0-5 (CIMMYT 2004)*

0	No Symptoms	The plant shows no visible symptoms of disease on the leaves. It is completely healthy, and there are no lesions or chlorotic areas present.
1	Very Slight Symptoms	Very small, isolated lesions are present, usually less than 1 cm in length. The lesions may be light chlorotic (yellowish) and do not show signs of necrosis (dead tissue). The disease is just beginning, and the plant remains largely unaffected.
2	Slight Symptoms	Small lesions (less than 2 cm) appear, often scattered across the leaves. These lesions may show light necrosis (brown or grayish tissue), but the overall leaf area affected is still relatively small. There is no significant impact on plant health.
3	Moderate Symptoms	Lesions are more numerous and may range from 2 to 4 cm in length. The necrosis is more visible, with larger areas of dead tissue. The plant may show some signs of reduced vigor, but the overall health of the plant is still acceptable.
4	Severe Symptoms	The lesions are large (greater than 4 cm in length) and numerous. The lesions often coalesce (merge), forming large necrotic areas. There is significant leaf damage, and the plant's photosynthetic capacity is noticeably reduced. Premature leaf death may occur.
5	Very Severe Symptoms	The plant shows widespread, severe necrosis across the leaf surface. Large lesions or coalesced lesions cover a large portion of the leaf area, and significant defoliation (early leaf death) may occur. The plant's growth and yield potential are severely impacted, and photosynthesis is greatly reduced.

Staining of leaf samples

Leaf samples were stained in 0.1% trypan blue solution, using lactoglycerol 1:1:1 (lactic acid: glycerol: H_2O) for 48 hours. Subsequently samples were washed in 1-chloral-hydratesolution (2.5g ml^{-1}) and left to set for 24 hours.

Screening of sample with light microscopy

Leaf samples were placed on glass slides with one drop of lactoglycerol and 0.1% phenol. A cover slip was placed over samples with a nail varnish and examined under light microscope (Labomed C×L, (HDCE-20 C, U.S.A).

Data collection

Data was recorded for each of the following histochemical parameters under light microscope.

Number of infections per microscopic field

Number of infections was calculated from three microscopic fields with the help of Labomed camera, fitted to light microscope. Average number of observed infections was calculated.

Fungal colony area of microscopic leaf samples

Fungal colony area (FCA) was recorded as per formula given by Baart et al. (1991).

$$FCA = Length \times Width \times \pi/4 \quad \text{(Eq.1)}$$

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Necrotic area (NA)

Necrotic area (NA) was calculated by using the following formula (Barakat et al., 2009).

$$NA = Length \times Width \times \pi/4 \quad \text{(Eq.2)}$$

Hypersensitivity index

Hypersensitivity index (HI) was calculated using the given formula (Baart et al., 1991).

$$HI = \frac{\text{Necrotic area}}{\text{Fungal colony area}} \times 100 \quad \text{(Eq.3)}$$

Statistical analysis

Fungal response was compared among compatible interaction of genotypes at10 and 20 dpi. Data were analyzed using descriptive statistics, range, minimum, maximum, Mean, variance using computer software SPSS 20. Graphics were made using MS Office Excel 2010. Fungal spore colonization was observed using Labomed C×L, (HDCE-20 C, U.S.A).

Results

Symptomology

Northern Corn Leaf Blight manifests as cigar-shaped lesions, colored grey-green or brown when mature. Lesions manifest parallel to the leaf surface. These lesions are grouped into susceptibility classes: (1) highly resistant, (2) resistant, (3) moderately resistant, (4) moderately susceptible, or (5) highly susceptible. Highly resistant genotypes developed no visible lesions on leaf surfaces (*Fig. 1a*), whereas resistant genotypes were characterized by little necrotic area with minute uredia (*Fig. 1b*). Moderately resistant genotypes had little-to-no uredia, but a clear covering of necrotized area was present. Moderately susceptible genotypes exhibited medium uredia but demonstrated no necrosis. Highly susceptible and moderately susceptible genotypes were classified with large uredia showing no chlorosis (*Fig. 1c, 1d*). The current study identified three highly susceptible, 3 highly resistant, 1 resistant, 14 moderately resistant and 9 moderately susceptible genotypes.

Histochemical studies per microscopic field

Fungal infection was clearly observed using histology. More infections were observed in susceptible lines (*Fig. 2a*). Number of infections per microscopic field varied between 0-22 at 10 dpi and 0–16 at 20 dpi per microscopic field. At 10 dpi, the highest infection count was reported in the Soan-3 (22 infections), then Ghuari (20), and Kissan (18). Of the strains that exhibited the lowest infection counts, few were observed in the resistant in NCEV-1530-11 strain (3), while no infections were observed in Karamat-Bar (0) and SZP-13200 (0) (*Fig. 2b*). At 20 dpi the situation had changed. Highest infection counts were still observed in Soan-3 (16), Ghuari (14) and Kissan (13) but an overall reduction

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in infection was observed in NCEV-1530-11 (2). Karamat-Bar and SZP-13200 continued to demonstrate no infections. The microscopic study confirmed the high susceptibility level of Soan-3, Ghuari, and Kissan when compared with their symptoms that were observed on leaf surface. In addition, resistance in the Karamat-Bar, SZP-13200 and NCEV-1530-11 was also confirmed by microscopic results when compared with their visual expression on leaf surface. The infections count observed microscopically in this study correspond with the results of the observed leaf symptoms under Northern corn leaf blight stress (*Table 2*).

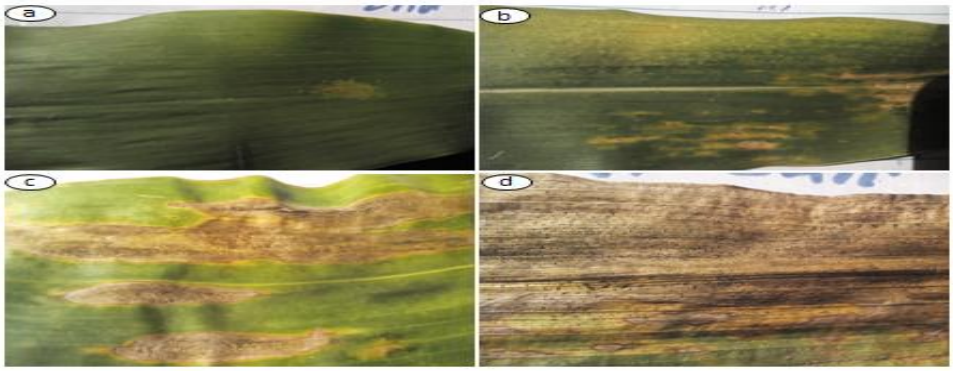

Figure 1. *No Number of infections, highly resistant (a), Resistant (b), Susceptible (c), Highly susceptible (d)*

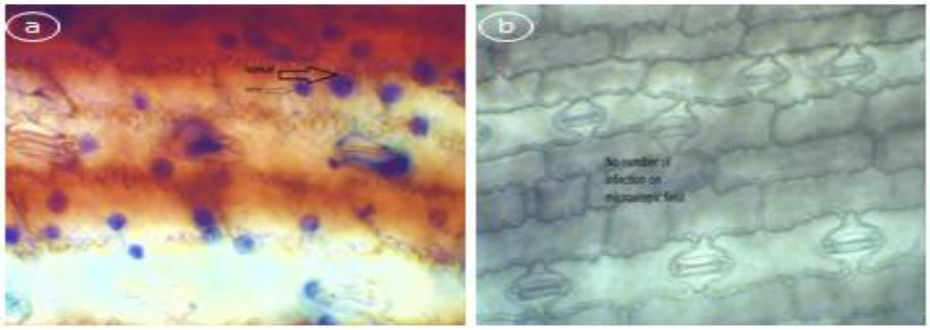

Figure 2. *(a) Large number of infections per microscopic field (NIPMF) in the maize genotype Soan-3 under NCLB (b) No number of infections in resistant genotype (Karamat-Bar)*

Necrotic area

Necrotic area (NA) at 10dpi ranged from 19.44-3456.6 µm. Maximum necrotic area was reported in Karamat-Bar (3456.66 µm), NCEV-1530-11 (726.57 µm), and SZP-13200 (625.68 µm) (*Figure 3a*). It was lowest in Soan-3 (19.44 µm), Ghuari (23.48 µm) and Kissan (40.43 µm). At 20 dpi, NA was reduced to a range of18.54-3354.45 µm. The resistant genotypes Karamat-Bar, NCEV-1530-11, Sarhed-white and SZP-13200 exhibited reduced necrotic area at 3354.45 µm, 721.34 µm, 543.22 and 534.00 µm, respectively and displayed the highest necrotic areas (*Figure 3b*) under microscopic field. These results are in agreement with the morphological symbolic response of genotypes against *E. turcicum.* The microscopic demonstration for high susceptibility genotypes

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Soan-3, Ghuari, and Kissan with low NA has also been agreed with the conferring response of genotypes under visual leaf surface observations.

Table 2. *Genotypes showing various symptoms conferring to Exserohilum turcicum on Maize leaf surface*

Genotypes	Symptoms conferring to *Exserohilum turcicum* on Maize leaf surface	Category
Karamat-Bar NCEV-1530-11 SZP-13200	No lesions, highly defense responses prevent pathogen spreading, no visible sign of disease, no necrosis, no premature death , no damage on photosynthetic area	Highly Resistant
NCEV-1530-4	No or very small lesions, effective defense mechanisms with localized cell death (hypersensitive response, HR), no premature leaf death, no spread of the disease and worthy photosynthetic area	Resistant
Ghuari Kissan Soan-3	Susceptible with large, elongated lesions, high lesion density, expansion, necrosis and premature leaf death	Highly Susceptible
Azam Sadaf Golden Iqbal-78 Pahari Aziz-2003 NCEV-1530-2 NCEV-1530-5 NCEV-1530-9 NCEV-1530-12 NARC-W NP-3 TP-1217 Rakaposhi	Smaller lesions or fewer lesions, less necrosis, slower spread of lesions across the leaf surface, lowering the overall damage, less necrosis**,** hypersensitive response (HR), infected cells die off rapidly to limit pathogen growth	Moderately Resistant
Jalal Sarhed-white NCEV-1530-1 NCEV-1530-3 NCEV-1530-6 NCEV-1530-7 NCEV-1530-10 NP-1 NP-2	Moderate lesion development, fewer and smaller lesions, slower lesion expansion, less premature leaf death, partial resistance responses**,** moderate damage reduce photosynthetic area	Moderately Susceptible

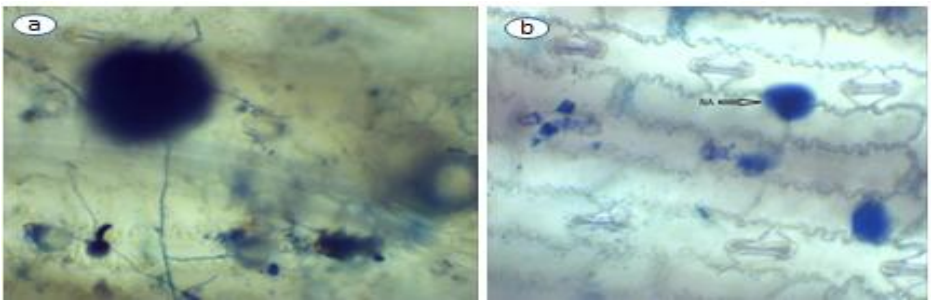

Figure 3. *(a) Large necrotic area (NA) detected in the genotypes (Karamat-Bar) R genotypes at 10 dpi (b) Necrotic area detected in the genotypes (Karamat-Bar) R genotypes at 20 dpi*

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Fungal colony area

At 10 dpi fungal colony areas (FCA) ranged 50.67-3943.04 µm. Greater fungal colony areas were observed in Soan-3 (3943.04 µm), Ghuari (2302.73 µm) and Kissan (1947.14 µm). In resistant genotypes, Karamat-Bar FCA was 49.45 µm, SZP-13200 was 52.34 µm, and NCEV-1530-11 was 95.45 µm. At 20 dpi, the fungus colonized widely, ranging 49.45-3911.2 µm. Genotypes Soan-3, Ghuari, and Kissan had reduced FCA to 3911.2 µm, 2202.73 µm and 1823.34 µm, respectively (*Figure 4b*). The reduction in 20 dpi showed that at initial stage of plant growth, pathogen spread more frequently as compared to days to 20 post inoculations.

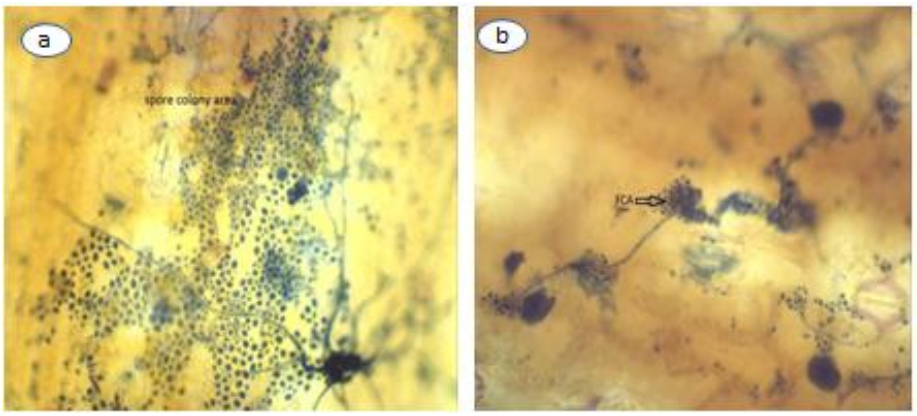

Figure 4. *(a) Fungal colony area (FCA) in maize leaf under microscopy. Genotype is Soan-3 (S) at 10 dpi (b) NCLB Fungal colony area (FCA) at 20 dpi in Soan-3*

Hypersensitivity index

The hypersensitive response is a plant defense response against pathogens in which plant cell necrosis is induced to control infection. This response is quantified using the hypersensitivity index (HI). Because necrotic area (NA) of host tissue exceeds the actual fungal colony area (FCA) in resistant genotypes, the hypersensitivity index gives a more accurate picture of immune response. At 10 dpi HI varied from 2.6% to 93.37% in resistant genotypes Karamat-Bar, SZP-13200, and NCEV-1530-11 at values of 93.37%, 93.09%, and 90.36%, respectively. Calculated HI values for susceptible genotypes were 2.6% in Soan-3, 2.51% in Ghuari, and 2.41% in Kissan (*Table 3*).

At 20 dpi the highest hypersensitivity index for Karamat-Bar, SZP-13200 and NCEV-1530-11 was determined to be 92.34%, 94.34% and 89.67% (Again, these genotypes have displayed highest resistance against *E. turcicum* and given indication of resistant gene against blighting The HI at 20 dpi was reduced in the genotypes susceptible reactions. In short, HI is maximum in highly resistant genotypes and minimum in susceptible ones. It reflects how the susceptible genetic response confers weak defense against fungal mycelium penetration of the epidermis, resulting in higher number of infections, greater spores, and larger colony areas. The current study was supported by findings of Camera et al. (2019). In contrast, deference mechanism conferred in resistant genotypes retard the infection by developing necrotic area barriers. These results are in agreement with the findings of Arif et al. (2018) (*Figure 5*).

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Table 3. *Histopathological traits of thirty maize genotypes at 10 and 20 dpi*

	NIMF		FCA		NA		HI	
Genotypes	**10dpi**	**20dpi**	**10dpi**	**20dpi**	**10dpi**	**20dpi**	**10dpi**	**20dpi**
Azam	10	7	1020.7	920.2	376.2	311.3	36.85	34.56
Ghuari	20	14	2302.73	2202.7	23.48	21.34	2.51	2.13
Jalal	16	11	946.56	842.43	238	211	25.14	24.34
Kissan	18	13	1947.14	1823.3	40.43	39.67	2.41	2.32
Sadaf	11	9	594.4	493.5	285.1	211.3	47.96	46.45
Sarhed-white	16	7	1658.75	1553.5	582.6	543.22	35.12	34.45
Golden	11	8	449.4	349.65	63.09	61.34	14.03	13.24
Iqbal-78	5	4	621	518.67	561.15	517.23	31.55	30.67
Karamat-Bar	0	0	50.67	49.45	3456.66	3354.4	93.37	92.34
Pahari	13	8	359.17	321.98	282.4	214.34	78.62	77.6
Aziz-2003	6	4	533.12	467.87	497.8	367.45	18.78	17.44
NCEV-1530-1	7	5	516.09	417.56	204.44	200.34	39.61	38.56
NCEV-1530-2	14	9	375.28	345.65	172.68	171.45	46.01	45.66
NCEV-1530-3	16	12	416.99	313.34	249.76	238.44	59.89	58.67
NCEV-1530-4	5	4	249.76	212.34	125.82	123.44	50.37	49.56
NCEV-1530-5	14	10	1315.94	1245.3	151.4	149.45	11.5	10.78
NCEV-1530-6	15	8	279.49	212.34	72.32	71.23	25.87	24.45
NCEV-1530-7	16	11	1786.2	1634.5	109.76	108.45	19.9	18.56
NCEV-1530-9	8	7	408	324	45.45	43.23	11.13	10.56
NCEV-1530-10	12	9	780.87	702.34	205.25	102.34	26.28	25.36
NCEV-1530-11	3	3	59.12	52.34	726.57	721.34	90.36	89.67
NCEV-1530-12	8	4	416.99	367.44	166.28	165.34	39.87	38.45
NARC-W	8	5	1171.09	1023.3	329.1	311.34	28.1	27.55
NP-1	16	9	968.6	845.3	591.78	516.45	61.09	60.88
NP-2	10	6	593.81	456.45	441.6	367.45	74.35	73.45
NP-3	9	5	416.016	323.34	387.28	323.23	32.13	31.44
TP-1217	14	9	369.36	222.34	209.03	201.45	56.59	55.56
SZP-13200	0	0	97.68	95.45	625.68	534	93.09	94.34
Rakaposhi	9	5	103.12	100.34	84.78	80.45	82.21	80.34
Soan-3	22	16	3943.04	3911.2	19.44	18.56	2.6	2
Mean	11.066		825.0362		377.5110		41.2430	
Minimum	.00		50.67		19.44		2.41	
Maximum	22.00		3943.04		3456.66		93.37	
Std. Deviation	5.5082		827.46358		615.2251		27.6939	
Variance	30.340		684695.97		378502.0		766.957	

NIMF=Number of infections per microscopic field, NA=Necrotic area, FCA= Fungal colony area, HI= Hypersensitive index, dpi= Days post infection

Descriptive statistics

The descriptive statistics for microscopic pathological traits (NIMF, FCA, NA, and HI) displayed in *Table 3*. Which seem to reflect the significance of variability of maize genotypes related to Northern Corn Leaf Blight (NCLB). Results showed that the range for NIMF varied between 0 to 22 indicates that the infection levels measured by this factor vary, with genotypes showing no/little measurable infection (0.00), while others have higher levels (up to 22). This suggests variability in how different genotypes or conditions respond to the disease. The average infection measurement is about 11, implying that, the

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plants show moderate infection. This is consistent with an environment where disease pressure is significant but not overwhelming. Standard Deviation for NIMF is 5.51 indicating a moderate standard deviation relative to the mean indicates that the infection measurements have substantial variability across the genotypes. This could be attributed to genetic differences, environmental factors, or varying pathogen loads, with some genotypes exhibiting resistance or tolerance while others show higher susceptibility. Variance for NIMF (30.34) showed that variance reinforces the standard deviation, showing a broad spread in infection levels across the sample population. It suggests that the population of genotypes has a diverse range of responses to the disease.

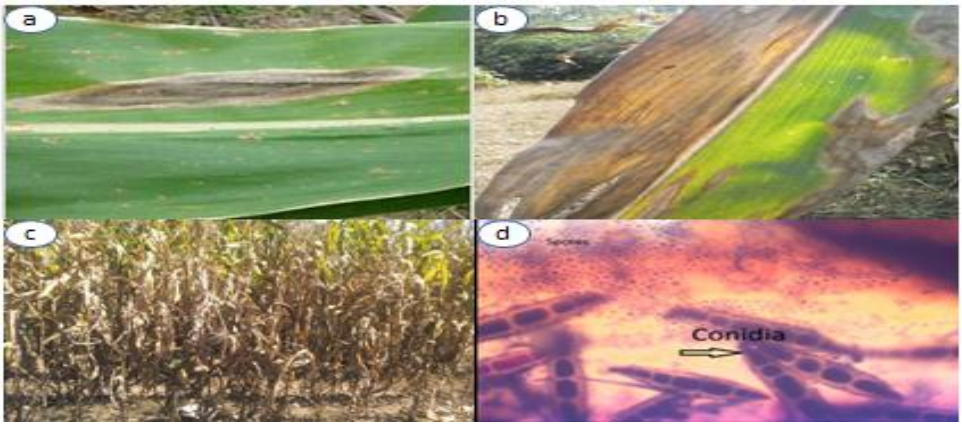

Figure 5. *a Necrotic lesion on maize leaf b. highly susceptible NCLB symptoms c. NCLB epidemics under artificial inoculation d. Conidia and spores of Exserohilum turcicum in infected leaf*

Results regarding FCA 'Range' varied from 50.67 to 3943.04 showed significant amount of variability in extent of fungal colonization. This suggests that some genotypes might be highly susceptible to the fungus, allowing for extensive fungal growth, while others may be more resistant or less favorable to fungal development. The average fungal colonization area is 825.04, which indicates a moderate level of fungal growth across the population. However, this mean is skewed by the large maximum value (3943.04), indicating that a small subset of plants may have extensive fungal colonization. This large variation implies that the genotypes are diverse in terms of fungal colonization. The high variance supports the high standard deviation, confirming that fungal colonization is very uneven across the germplasm. It could be useful to explore what factors (such as genotype, environment, or treatments) contribute to this variability. The wide range of necrosis area suggests that there are substantial differences in the extent of tissue damage caused by the infection. The minimum value (19.44) indicates minimal necrosis in some genotypes, while the maximum (3456.66) shows that other genotypes exhibit severe damage. Mean value (377.51) indicated that the necrosis area is relatively small compared to the maximum value, suggesting that while most genotypes might show some degree of necrosis, it doesn't necessarily extend to severe levels across the entire germplasm. The high standard deviation relative to the mean reflects a high level of variability in tissue damage. Some genotypes showed minimal necrosis, while others experience extensive damage. This variability could point to different resistance levels among the genotypes or potentially the timing of infection or severity of the pathogen. The high variance is consistent with the high standard deviation, suggesting that necrosis is highly variable

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amongst germplasm. Further investigation could explore factors contributing to this range, such as genotype differences in susceptibility or differences in infection timing and environmental conditions.

Data regarding to hypersensitivity index shows variability in the genotypes defense responses to the infection. A minimum of 2.41 suggests that susceptible genotypes may have low or no hypersensitive response, while the maximum value of 93.37 indicates a strong defense response in resistant genotypes. The mean value of 41.24 suggests a moderate average hypersensitive response across the germplasm, indicating that, on average, the genotypes show some level of defense mechanisms in response to the infection. The standard deviation of 27.69 indicates substantial variability in the hypersensitive responses. This could be linked to genetic diversity among the genotypes, where some are capable of mounting a strong defense while others show weaker responses. Variance further confirms the high spread in hypersensitivity responses. This suggests that while resistant genotypes may show robust defense mechanisms, others may be more susceptible or have a weaker response to the pathogen.

Discussion

Histopathological traits indicated that maize genotypes Karamat-Bar, SZP-13200, and NCEV-1530-11 had least number of infections (NI), lowest fungal colony area (FCA), and demonstrated resistant infection response at both disease assessment/symptoms expression and microscopic observations. This supports the presence of the Ht resistance gene in these genotypes. The cells of resistant genotypes have been characterized by vessel enlargement along the site of infection preventing the progress of the NCLB pathogen (Carson, 2006) resulting in less sporulation and inhibiting fungal colony formation (Miedaner and Garbelotto, 2024). The genotypes Soan-3, Ghuari, and Kissan had the highest NIs and largest FCA confirming their susceptible response against *E. turcicum*. These susceptible genotypes showed no resistance response, which is manifested in cell/tissue death. As no spots were observed microscopically with trypan blue application, it can be surmised that there was no resistance response. Susceptible genotypes exhibited little-to-no necrosis, greater FCA, and lower HI values. This correlates with previous research describing resistance to NCLB as being expressed histopathological in xylem tissue. The pathogen spreading early, exhibiting susceptible reaction with mycelial walls progressing towards the tracheid (Fang et al., 2022). Both lateral and longitudinal growth in hyphae is reduced in resistant varieties; resistant host responses also inhibit mycelia formation. As a result, necrosis and lesion progression was slow (Galiano-Carneiro and Miedaner, 2017). Hyphal branches are smaller in resistant varieties, with hindered necrosis and smaller leaf lesions in resistant genotypes (Hooda et al., 2017).

Many classes of hypersensitive response have been studied in this experiment, as quantified by HI. Highly resistant genotypes demonstrated larger HI response to *E. turcicum* then susceptible ones. Necrotic area (NA) of host tissue exceeded actual FCA in resistant, high HI genotypes. The interveinal penetration of *E. turcicum* in the maize leaf caused the death of leaf sheath in susceptible cultivars (Kang et al., 2002). They reported that hypersensitivity cell death occurs with incompatible interactions between host tissue and hyphal growth with the number of haustoria greatly reduced as a result (when compared to compatible interactions in susceptible tissue). Hypersensitive necrosis is a complex phenomenon which plays a significant role in host resistance response

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(Kotze et al., 2019). Sorensen (2017) found that presence of necrotic area per fungal colony was correlated with resistant response. Research continues to demonstrate hypersensitive necrosis and HI as key indicators of maize resistance to infection Histochemical evaluation of highly resistant and highly susceptible maize genotypes revealed the importance of resistance in selection (Ohunakin et al., 2019a). In this study, the confirmations of resistance in maize gene pool were characterized by presence of necrotic area, restriction of fungal colony area and highest hypersensitivity index. This would helpful for exploitation of new improved genetic material against NCLB stress and helpful in reduction of yield losses. The results are in agreement with the findings of Bellincampi et al. (2014).

In addition, the variability across all variables (NIMF, FCA, NA, HI) suggests diverse group of genotypes, ranging from resistant to susceptible. Genotypes showing low FCA and NA (fungal colonization and necrosis area) and high HI (hypersensitivity) likely have effective resistance mechanisms. Conversely, those with high FCA and NA, combined with low HI, are more susceptible to the disease. The large spread in the data for each variable implies that different maize genotypes respond differently to NCLB, with some being able to limit pathogen growth and tissue damage more effectively than others. This could have implications for breeding programs aimed at selecting resistant maize varieties (Opio et al., 2010). Besides genetic differences, environmental factors (such as humidity, temperature, and field conditions) may also contribute to the observed variability in infection severity and defense response (Mubeen et al., 2017; Desoky et al., 2020; Elrys et al., 2020). Pathogen load and the timing of infection could influence the degree of fungal colonization and necrosis (Wisser et al., 2006). Future studies could focus on identifying the specific genetic traits associated with resistance to NCLB, investigating the underlying molecular mechanisms of hypersensitive responses, and understanding how environmental factors influence disease dynamics. Additionally, identifying how to reduce the high variability in FCA and NA, especially in susceptible genotypes, could lead to more targeted disease management strategies. It is concluded from the current findings, the germplasm showed a significant amount of variability which could helpful for integrated disease management approaches considering both genetic and environmental factors. It also provides information to understanding, how maize genotypes respond to NCLB and indicating a potential for developing resistant genotypes through various breeding strategies.

Conclusion

This study has concluded that the fungus produced higher infection counts and greater fungal colony areas in highly susceptible maize genotypes (Soan-3, Kissan, Ghuari) as compared to highly resistant genotypes (Karamat-Bar, SZP-13200, NCEV-1530-11). In addition, necrotic area was greater in resistant and moderately resistant strains, slowing the progression of leaf blight as measured by HI indicating the presence of certain resistant genes. The highest HI values in resistant genotypes indicates strong host defense against NCLB disease stress. The identified resistant genetic material could be exploiting for future breeding programs.

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REFERENCES

[1] Arif, M. J., Wakil, W., Gogi, M. D., Khan, R. R., Arshad, M., Sufyan, M., Nawaz, A., Ali, A., Majeed, S. (2018): Trends in sustainable management of emerging insect pests. – In: Khan, I. A., Khan, M. S. (eds.): Developing Sustainable Agriculture in Pakistan, pp. 417-484. CRC Press, Boca Raton.

[2] Baart, P. G. J., Parlevliet, J. E., Limburg, H. (1991): Effects of infection density on the size of barley and wheat leaf rust colonies before and on the size of uredia after the start of sporulation. – J. Phytopathol. 131: 59-64. 10.1111/j.1439-0434.1991.tb04571.x.

[3] Balint-Kurti, P. (2019): The plant hypersensitive response: concepts, control and consequences. – Molecular Plant Pathology 20: 1163-1178.

[4] Barakat, M. N., El-Shafei, A. A., Al-Doss, A. A. (2009): Identification of molecular markers linked to northern corn leaf blight resistance in yellow population of maize. – G3: Genes/Genomes/Genet 3: 89-95.

[5] Bellincampi, D., Cervone, F., Lionetti, V. (2014): Plant cell wall dynamics and wall-related susceptibility in plant–pathogen interactions. – Frontiers in Plant Science 5: 87127.

[6] Camera, J. N., Forcelini, C. A., Koefender, J., Golle, D. P., Schoffel, A., Deuner, C. C. (2019): Reação de híbridos de milho a helmintosporiose e ferrugem comum, e controle químico de helmintosporiose. – Arquivos do Instituto Biológico 86: e0082018.

[7] Carson, M. (2006): Response of a maize synthetic to selection for components of partial resistance to *Exserohilum turcicum*. – Plant Disease 90: 910-914.

[8] CIMMYT. (2004): Maize disease. A guide for field identification. – 4th ed. Government of Pakistan. Minist. Food, Agric. Livestock, Econ. Wing. Islamabad, Pakistan.

[9] Cooksey, C. J. (2014): Quirks of dye nomenclature. 3. Trypan blue. – Biotech Histochem 89(8): 564-567.

[10] Degani, O., Regev, D., Dor, S. (2021): The microflora of maize grains as a biological barrier against the late wilt causal agent, *Magnaporthiopsis maydis*. – Agronomy 11: 965.

[11] Desoky, E. S. M., Maghraby, L. M., El Awad, A. E., Abdo, A. I., Rady, M. M., Semida, W. M. (2020): Fennel and ammi seed extracts modulate antioxidant defence system and alleviate salinity stress in cowpea (*Vigna unguiculata*). – Scientia Horticulturae 272: 109576.

[12] El-Baky, N. A., Al Fattah Amara, A. A. (2021): Recent approaches towards control of fungal diseases in plants: An updated review. – Journal of Fungi 7: 900.

[13] Elrys, A. S., Desoky, E. S. M., El-Maati, M. F. A., Elnahal, A. S., Abdo, A. I., Raza, S., Zhou, J. (2019): Can secondary metabolites extracted from Moringa seeds suppress ammonia oxidizers to increase nitrogen use efficiency and reduce nitrate contamination in potato tubers? – Ecotoxicology and Environmental Safety 185: 109689.

[14] Fang, Y. L., Zhou, Y. Y., Li, X., Gao, Y., Wang, D. L., Liu, M. J., Zhang, Z. J. (2022): Histological characterization of the early-stage infection events of *Setosphaeria turcica* in maize. – Plant Pathology 71: 251-261.

[15] Fathima, S. D., Gururaj, N., Sivapathasundharam, B., Vennila, A. A., Lavanya, M. K., Sarayushivani, U. (2023): Histopathological significance of necrosis in oral lesions: A review. – Journal of Oral and Maxillofacial Pathology 27: 340-347.

[16] Galiano-Carneiro, A. L., Miedaner, T. (2017): Genetics of resistance and pathogenicity in the maize/*Setosphaeria turcica* pathosystem and implications for breeding. – Frontiers in Plant Science 8: 283888.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_66896703

[17] Haque, M. A., Marwaha, S., Deb, C. K., Nigam, S., Arora, A., Hooda, K. S., Soujanya, P. L., Aggarwal, S. K., Lall, B., Kumar, M. (2022): Deep learning-based approach for identification of diseases of maize crop. – Scientific Reports 12: 6334.
[18] Hooda, K., Khokhar, M., Shekhar, M., Karjagi, C. G., Kumar, B., Mallikarjuna, N., Devlash, R., Chandrashekara, C., Yadav, O. (2017): Turcicum leaf blight-sustainable management of a re-emerging maize disease. – Journal of Plant Diseases and Protection 124: 101-113.
[19] Hotz, E. C., Bradshaw, A. J., Elliott, C., Carlson, K., Dentinger, B. T., Naleway, S. E. (2023): Effect of agar concentration on structure and physiology of fungal hyphal systems. – Journal of Materials Research and Technology 24: 7614-7623.
[20] Kang, Z., Huang, L., Buchenauer, H. (2002): Ultrastructural changes and localization of lignin and callose in compatible and incompatible interactions between wheat and *Puccinia striiformis*/Ultrastrukturelle veränderungen und lokalisierung von lignin und kailose in kompatiblen und inkompatiblen interaktionen zwischen weizen und Puccinia striiformis. – Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection 109(1): 25-37.
[21] Kotze, R., Van der Merwe, C., Crampton, B., Kritzinger, Q. (2019): A histological assessment of the infection strategy of *Exserohilum turcicum* in maize. – Plant Pathology 68: 504-512.
[22] Mideros, S. X., Chung, C.-L., Wiesner-Hanks, T., Poland, J. A., Wu, D., Fialko, A. A., Turgeon, B. G., Nelson, R. J. (2018): Determinants of virulence and in vitro development colocalize on a genetic map of *Setosphaeria turcica*. – Phytopathology 108: 254-263.
[23] Miedaner, T., Garbelotto, M. M. (2024): Human-mediated migration of plants, their pathogens and parasites. – Journal of Plant Pathology 106: 301-325.
[24] Mubeen, S., Rafique, M., Munis, M. F. H., Chaudhary, H. J. (2017): Study of southern corn leaf blight (SCLB) on maize genotypes and its effect on yield. – Journal of the Saudi Society of Agricultural Sciences 16(3): 210-217.
[25] Ohunakin, A., Odiyi, A., Akinyele, B. (2019a): Comparison of rank sum and Area under Disease Progress Curve (AUDPC) as determinant for relative resistance status of maize populations to Northern leaf blight disease of maize. – Adv Plants Agric Res 9: 395-400.
[26] Ohunakin, F., Adeniji, A. A., Oludayo, O. A., Osibanjo, A. O., Oduyoye, O. O. (2019b): Employees' retention in Nigeria's hospitality industry: The role of transformational leadership style and job satisfaction. – Journal of Human Resources in Hospitality & Tourism 18: 441-470.
[27] Opio, L., Asea, G., Baguma, Y., Okori, P. (2010): Heterosis and combining abilities for multiple resistance to Turcicum leaf blight and maize streak virus. – RUFORUM Second Biennial Conference, Entebbe, Uganda, 20-24 September 2010.
[28] Reddy, T. R., Reddy, P. N., Reddy, R. R. (2013): Turcicum leaf blight of maize incited by *Exserohilum turcicum*: A review. – International Journal of Applied Biology and Pharmaceutical Technology 5: 54-60.
[29] Rehman, U. F., Adnan, M., Kalsoom, M., Naz, N., Husnain, M. G., Ilahi, H., Ilyas, M. A., Yousaf, G., Tahir, R., Ahmad, U. (2021): Seed-borne fungal diseases of maize (*Zea mays* L.): A review. – Agrinula: Jurnal Agroteknologi dan Perkebunan 4: 43-60.
[30] Smith, C. W., Betrán, J., Runge, E. C. (2004): Corn: origin, history, technology, and production. – John Wiley & Sons.
[31] Sørensen, C. K., Labouriau, R., Hovmøller, M. S. (2017): Temporal and spatial variability of fungal structures and host responses in an incompatible rust–wheat interaction. – Frontiers in Plant Science 8: 254526.
[32] Wise, K. A., Smith, D., Freije, A., Mueller, D. S., Kandel, Y., Allen, T., Bradley, C. A., Byamukama, E., Chilvers, M., Faske, T. (2019): Meta-analysis of yield response of foliar fungicide-treated hybrid corn in the United States and Ontario, Canada. – PLoS One 14: e0217510.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_66896703

[33] Wisser, R. J., Balint-Kurti, P. J., Nelson, R. J. (2006): The Genetic Architecture of Disease Resistance in Maize: A Synthesis of Published Studies. – Phytopathology 96(2): 120-129.
[34] Zhu, M., Tong, L., Xu, M., Zhong, T. (2021): Genetic dissection of maize disease resistance and its applications in molecular breeding. – Molecular Breeding 41: 32.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2304_66896703