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Research Article

Field screening of linseed genotypes against alternaria blight (*Alternaria lini*)

Subash Subedi[1*], Saraswati Neupane[2], Sailendra Yadav[1] and Jiban Shrestha[3]

[1]Oilseed Research Program, Nawalpur, Sarlahi, Nepal
[2]National Maize Research Program, Rampur, Chitwan, Nepal
[3]National Plant Breeding and Genetics Research Centre, Khumaltar, Lalitpur, Nepal
[*]Correspondence: subedi.subash1@gmail.com
[*]ORCID: https://orcid.org/0000-0003-3739-1773

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ABSTRACT

Linseed (*Linum usitatissimum* L.) is a promising but underexplored oilseed crop in Nepal, with considerable expansion potential. However, it faces a significant threat from leaf blight disease, caused by *Alternaria lini* resulting in notable yield reductions. This study aimed to identify genetic sources of resistance to this pathogen. Fourty-two linseed genotypes, including resistant check ACC#7622 and susceptible check Sarlahi local, underwent disease resistance screening over two years (2021-2022) in field disease screening nurseries at the Oilseed Research Program, Sarlahi, using an incomplete block design. Each block contained seven genotypes, replicated twice. Disease severity, yield and yield attributing traits were assessed. Significant variations in disease and yield traits were observed among the genotypes over the years. While only 19% showed moderate resistance with minimal symptoms compared to susceptible types. None were immune and resistance, with 13 genotypes (31%) classified as moderately susceptible, 26% susceptible and 24% highly susceptible. A significant negative correlation ($P \leq 0.05$) between disease severity and grain yield was evident. Genotypes ACC#(5-ICLI-2001-5), ACC#96-001, ACC#7622, ACC#96-004, TN#04, ACC#(9-ICLI-2001-9), ACC#(1-ICLI-2001-1), and TN#08 demonstrated both high yield and moderately resistance to alternaria leaf blight, offering valuable genetic resources for enhancing linseed resilience to this disease through breeding initiatives.

Keywords: *Alternaria lini*, Diseases severity, Leaf blight, Linseed, Resistance

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INTRODUCTION

Linseed (*Linum usitatissimum* L.) (2n=30) belongs to family Linaceae and genus Linum is an economically important oilseed and fiber crop widely cultivated for its high-quality oil, rich in alpha-linolenic acid, and for its application in textiles, pharmaceuticals, and other industries (Katare *et al*., 2012). It ranked as the third most important oilseed crop in Nepal, following rapeseed and mustard. In the 2021/22 season, oilseed crops were cultivated on 260,645 hectares, yielding 287,344 metric tons with an average productivity of 1.10 metric tons per hectare. Linseed accounted for 12,856 hectares, producing 12,923 metric tons at a productivity of 1.01 metric tons per hectare (MoALD, 2023). It plays a significant role in the agricultural economy of Nepal, particularly in terai-plane regions, where it is commonly

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grown under diverse agro-climatic conditions (Subedi, 2023). However, the productivity of linseed is frequently challenged by several biotic stresses, among which Alternaria blight, caused by *Alternaria lini*, is a major concern (ORP, 2017). Alternaria blight is a devastating fungal disease that significantly reduces yield and seed quality (Singh, 2003). The disease is characterized by the formation of brown necrotic lesions with concentric rings on leaves, stems, and capsules, leading to premature defoliation, pod abortion, and ultimately poor seed development (Singh and Singh, 2005). The warm and humid climatic conditions prevalent in many linseed-growing regions of Nepal, provide a conducive environment for the rapid proliferation of this pathogen (Subedi, 2023).

Although cultural practices and chemical control measures can mitigate the impact of Alternaria blight, these approaches are often unsustainable due to economic constraints and environmental concerns (Singh, 2003). Therefore, developing and identifying resistant or tolerant genotypes through field screening is a critical strategy for managing this disease. Screening under field conditions allows for the evaluation of genotypes in a natural infection environment, providing valuable insights into their performance and resistance under natural epiphytotic conditions (Subedi, 2015). The present study was undertaken to evaluate 42 linseed genotypes for their resistance to Alternaria blight under field conditions in Sarlahi, Nawalpur, Nepal. The objective was to identify promising genotypes that could serve as a resource for breeding programs or be directly adopted by farmers in regions prone to Alternaria blight outbreaks. This research contributes to sustainable disease management and supports efforts to enhance linseed productivity in Nepal.

MATERIALS AND METHODS

The study evaluated 42 linseed genotypes, including ACC#7622 as a resistant check and Sarlahi Local as a susceptible check, under natural infestation conditions using an incomplete block (alpha lattice) design during the winter seasons of 2021 and 2022. The experiment comprised six blocks, each containing seven genotypes replicated twice. The field trials were conducted at the Oilseed Research Program (ORP) in Nawalpur, Sarlahi, Nepal, located at 27°03'86" N latitude and 85°35'52" E longitude, at an elevation of 144 masl. The experimental site features slightly acidic (pH 4.5–6) sandy loam soil, with 1.34% organic matter, low nitrogen content, medium potassium levels, and high phosphorus availability (ORP, 2021).

The genotypes were sourced from national and international origins and maintained within the breeding program at ORP, Nawalpur. Each unit plot measured 4 m × 2 × 0.35 m, consisting of two rows spaced 35 cm apart, resulting in a net harvested area of 2.8 m². Planting was performed in the last week of October in both years, following a recommended fertilizer application of 60:40:20 kg/ha of $N:P_2O_5:K_2O$, supplemented with 8 t/ha of farmyard manure (FYM), and a seed rate of 30 kg/ha (ORP, 2017). Standard cultural and agronomic practices were implemented as per institutional guidelines (ORP, 2017).

Data on Alternaria leaf blight disease severity was recorded from five randomly tagged plants per plot using a 0-5 scoring scale developed by Singh and Singh (2005).

- 0 = No infection
- 1 = 1-10% leaf area affected at lower parts of the plant
- 2 = 10.1-25% leaf area affected
- 3 = 25.1-50% leaf area affected

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- 4 = 50.1-75% leaf area affected
- 5 = 75.1-100% leaf area affected

The percent disease index (PDI) was calculated using Wheeler's (1969) formula:

$$\text{PDI (\%)} = \frac{(Sum\ of\ all\ numerical\ values)}{(No\ of\ plants\ observed\ \times\ Maximum\ diseases\ rating)} \times 100 \quad \text{……………..(1)}$$

Based on the PDI, disease reactions were categorized as follows (ORP, 2017):

- 0% = Immune
- 1-10% = Resistant (R)
- 10.1-25% = Moderately resistant (MR)
- 25.1-50% = Moderately Susceptible (MS)
- 50.1-75% = Susceptible (S)
- 75.1-100% = Highly susceptible (HS)

Yield and yield-attributing traits, including days to flowering, days to maturity, plant height (cm), pods per plant, seeds per pod, grain yield (kg/ha) were recorded (ORP, 2017).

Statistical analysis was performed using Microsoft Excel 2013 and GENSTAT 18th edition. The relationship between the disease index and grain yield was also assessed.

RESULTS AND DISCUSSION

Linseed genotypes varied significantly (P≤0.05) for days to flowering, days to maturity, plant height (cm), pod per plant, seed per pod, grain yield (kg/ha) and alternaria leaf blight severity during 2021 (Table 1). The days to flowering ranged (69.5-78) with the mean average of 73.33 ± 0.27, days to maturity (134.5-143) with the mean average of 139.37 ± 0.26, plant height (42.33-61.13 cm) with the mean average of 53.75 ± 0.45 cm, Pod per plant (10.80-48.25) with the mean average of 30.17 ± 1.08, seed per pod (5.20-8.90) with the mean average of 7.04 ± 0.16, grain yield (311.17-883.31 kg/ha) with the mean average of 683.20 ± 13.88 and alternaria leaf blight severity (14-90 %) with the mean average of 52.55 ± 2.47 % (Table 1).

Table 1. Statistical analysis of yield components, yield and alternaria leaf blight severity in linseed genotypes evaluated at ORP, Sarlahi, Nepal during 2021

Parameters	Mean ± SEm	Range	P-value	LSD (0.05)	CV,%
Days to Flowering	73.33 ± 0.27	69.5-78	<.001	1.43	0.96
Days to maturity	139.37 ± 0.26	134.5-143	<.001	1.58	0.56
Plant height (cm)	53.75 ± 0.45	42.33-61.13	<.001	0.01	0.01
Pod per plant	30.17 ± 1.08	10.80-48.25	<.001	0.16	0.26
Seed per pod	7.04 ± 0.16	5.20-8.90	<.001	0.41	2.89
Grain Yield (kg/ha)	683.20 ± 13.88	311.17-883.31	<.001	18.92	1.37
Disease severity (PDI %)	52.55 ± 2.47	14-90	<.001	4.05	3.80

In 2022, similar trends were observed, showing statistically significant differences in all recorded parameters except seed per pod among the tested linseed genotypes (Table 2). The parameters and their ranges were as follows: days to flowering (33-52, mean 42.98 ± 0.34), days to maturity (89-107, mean 97.74 ± 0.32), plant height (99-192 cm, mean 145.41 ± 1.37 cm), pods per plant (16-83, mean 43.01 ± 1.15), seeds per pod (3-24, mean 7.04 ± 0.36),

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grain yield (500-1123 kg/ha, mean 753.42 ± 14.60 kg/ha) and alternaria leaf blight severity (20-92%, mean 55.184 ± 1.80%) (Table 2).

Table 2. Statistical analysis of yield components, yield and alternaria leaf blight severity in linseed genotypes evaluated at ORP, Sarlahi, Nepal during 2022

Parameters	Mean ± Sem	Range	P-value	LSD (0.05)	CV,%
Days to Flowering	81.56 ± 0.40	77-87.5	<.001	0.98	0.59
Days to maturity	130.05 ± 0.44	124-135.5	<.001	1.19	0.45
Plant height (cm)	54.79 ± 0.44	42-61	<.001	1.88	1.69
Pod per plant	23.06 ± 0.60	11-36.5	<.001	2.43	5.2
Seed per pod	8.08 ± 0.20	5.5-10.5	0.126	3.24	19.76
Grain Yield (kg/ha)	700.69 ± 9.62	503.5-857.3	<.001	22.23	1.56
Disease severity (PDI %)	51.60 ± 2.44	14-90	<.001	5.35	5.12

The combined mean performance of linseed genotypes for the alternaria leaf blight disease severity, yield and yield components during 2021-2022 shown in Table 3. Statistically highly significant differences were observed for the parameters days to flowering, days to maturity, plant height (cm), pod per plant, seed per pod, grain yield (kg/ha) and alternaria leaf blight severity among the tested linseed genotypes in combined analysis for two consecutive years.

Table 3. Combined mean performance of linseed genotypes to the yield, yield components and alternaria leaf blight disease severity during 2021-2022 at Nawalpur, Sarlahi, Nepal

Genotypes	FLD	Mat D	Pht (cm)	Pod /Pl	S/P	GY (kg/ha)	PDI %
ACC#96005	74.25[†]	130.25	46.87	12.00	8.00	594.92	84.00
ACC#8070	77.25	132.75	52.07	13.15	7.60	545.42	78.00
TN#01	81.25	134.25	52.47	19.95	5.85	559.19	84.00
ACC#6103	79.75	133.75	55.77	17.45	7.00	754.43	40.00
ACC#9603	76.25	136.25	49.67	20.85	7.20	623.46	78.00
ACC#8660	80.25	134.50	51.37	18.05	8.10	689.91	28.00
ACC#8656	78.25	137.50	52.97	18.75	6.85	610.94	86.00
ACC#8657	79.25	133.00	56.62	27.08	7.05	634.77	84.00
ACC#96001	78.75	135.00	56.62	14.88	7.15	671.07	66.00
ACC#97003	80.75	134.00	57.52	20.83	7.40	703.66	50.00
ACC#7553	79.75	133.25	48.92	24.73	8.00	720.25	42.00
ACC#8069	79.25	137.75	55.02	29.03	8.70	676.66	58.00
ACC#8654	80.00	137.75	48.72	24.03	9.70	722.63	44.00
ACC#8658	78.50	136.25	55.22	25.33	8.10	686.53	60.00
TN#02	77.00	134.50	55.52	27.99	8.60	670.78	60.00
ACC#(9-ICLI-2001-9)	75.25	132.00	56.77	35.49	7.98	761.06	24.00
TN#03	75.25	131.00	50.47	24.09	7.68	710.80	44.00
ACC#(1-ICLI-2001-1)	75.75	133.00	58.97	30.79	7.63	865.55	24.00
TN#04	74.75	133.00	55.77	26.99	6.83	790.51	21.00
ACC#(5-ICLI-2001-5)	74.25	132.50	49.67	28.29	8.03	773.13	14.00
TN#05	75.25	136.00	55.62	31.99	8.63	679.41	58.00
ACC#(8-ICLI-2001-8)	76.75	136.50	58.22	33.49	8.08	681.69	52.00
ACC#(3-ICLI-2001-3)	78.25	137.25	56.92	24.79	7.78	734.68	38.00
ACC#(4-ICLI-2001-4)	78.25	136.25	54.52	29.99	6.28	732.43	30.00

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Genotypes	**FLD**	**Mat D**	**Pht (cm)**	**Pod /Pl**	**S/P**	**GY (kg/ha)**	**PDI %**
ACC#97001	80.25	137.50	56.72	33.09	6.63	731.43	38.00
ACC#8652	77.75	138.00	57.02	27.88	6.73	614.59	82.00
ACC#96-002	73.75	137.00	56.42	31.98	7.63	666.97	60.00
ACC#7704	81.00	136.50	56.02	30.93	7.13	639.29	62.00
ACC#96-001	78.50	135.50	51.72	31.43	7.23	784.96	16.00
TN#06	74.50	132.25	52.92	21.48	6.33	609.57	78.00
TN#07	80.25	137.75	53.42	20.09	8.28	634.83	70.00
ACC#96-004	77.75	133.25	55.72	32.44	8.55	782.45	18.00
ACC#7345	75.75	132.25	58.72	29.94	8.35	758.43	42.00
ACC#6104	74.75	133.00	57.82	33.69	7.78	707.69	50.00
TN#08	75.25	134.00	57.92	25.45	8.28	789.16	24.00
TN#09	75.25	137.50	55.27	27.15	6.13	676.72	60.00
ACC#97-005	80.75	138.50	53.87	25.10	7.03	657.19	62.00
ACC#97-006	80.00	137.00	52.97	32.93	7.03	617.07	88.00
Aust CULT PBR Ninola	75.00	130.00	53.17	35.33	6.63	739.39	50.00
Aust CULT PBR NinolaWellanga	74.00	130.50	48.87	35.63	8.05	727.59	48.00
ACC#7622 (RC)	**78.75**	**136.00**	**52.82**	**32.24**	**8.08**	**799.17**	**16.00**
Sarlahi Local (SC)	**75.25**	**133.00**	**55.77**	**31.09**	**7.60**	**531.49**	**76.00**
Grand mean	77.45	134.71	54.27	26.61	7.56	691.95	52.07
Min	73.75	130.00	46.87	12.00	5.85	531.49	14.00
Max	81.25	138.50	58.97	35.63	9.70	865.55	88.00
CV, %	1.92	0.77	1.54	3.82	15.86	2.31	5.01
LSD (0.05)							
Genotype (G)	2.10	1.47	1.17	1.43	1.69	22.48	3.67
Year (Y)	0.46	0.32	0.26	0.31	0.37	4.91	0.80
G × Y	2.96	2.07	1.66	2.03	2.39	31.79	5.20
P-value							
Genotype (G)	<.001	<.001	<.001	<.001	0.018	<.001	<.001
Year (Y)	<.001	<.001	<.001	<.001	<.001	<.001	<.001
G × Y	<.001	<.001	<.001	<.001	0.02	<.001	<.001

[†]Means of 2 replications, FL_D-days to flowering, Mat_D-days to maturity, Pht (cm)-plant height in centimeter, Pod/PL-Pod per plant, PDI-percent disease index, GY-grain yield, kg/ha-kilogram per hectare, RC-resistant check, SC- susceptible check

The parameters and their ranges in combined analysis were as follows: days to flowering (73.75-81.25, mean 77.45), days to maturity (130-138.5, mean 134.71), plant height (46.87-58.97 cm, mean 54.27 cm), pods per plant (12-35.63, mean 26.61), seeds per pod (5.85-9.70, mean 7.56), grain yield (531.49-865.55 kg/ha, mean 691.95 kg/ha) and alternaria leaf blight severity (14-88%, mean 52.07 %) (Table 3). The lower percent disease index and higher yield were recorded in genotypes ACC#5-ICLI-2001-5 (PDI-14% and GY-773.13 kg/ha), ACC#96-001 (PDI-16% and GY-784.96 kg/ha), ACC#7622 (PDI-16% and GY-799.17 kg/ha), ACC#96-004 (PDI-18% and GY-782.45 kg/ha), TN#04 (PDI-21% and GY-790.51 kg/ha) and ACC#9-ICLI-2001-9 (PDI-24% and GY-761.06 kg/ha), ACC#1-ICLI-2001-1 (PDI-24% and GY-865.55 kg/ha) and TN#08 (PDI-24% and GY-789.16 kg/ha) respectively (Table 3).

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Table 4. Reaction of linseed genotypes against alternaria leaf blight disease during 2021-2022 at Nawalpur, Sarlahi, Nepal

Disease reaction	Severity	Genotypes
Immune (I)	0%	Nil
Resistant (R)	1-10%	Nil
Moderately resistant (MR)	10.1-25%	ACC#(5-ICLI-2001-5), ACC#96-001, ACC#7622, ACC#96-004, TN#04, ACC#(9-ICLI-2001-9), ACC#(1-ICLI-2001-1), TN#08
Moderately Susceptible (MS)	25.1-50%	ACC#8660, ACC#(4-ICLI-2001-4), ACC#(3-ICLI-2001-3), ACC#97001, ACC#6103, ACC#7553, ACC#7345, ACC#8654, TN#03, Aust CULT PBR Ninola Wellanga, ACC#97003, ACC#6104, Aust CULT PBR Ninola
Susceptible (S)	50.1-75%	ACC#(8-ICLI-2001-8), ACC#8069, TN#05, ACC#8658, TN#02, ACC#96-002, TN#09, ACC#7704, ACC#97-005, ACC#96001, TN#07
Highly susceptible (HS)	75.1-100%	Sarlahi Local, ACC#8070, ACC#9603, TN#06, ACC#8652, ACC#96005, TN#01, ACC#8657, ACC#8656, ACC#97-006

Relationship between disease index (PDI) and grain yield

The best fit, with adjusted R^2 = 83%, showed a significant ($P \leq 0.05$) linear negative correlation (r=-0.91) between grain yield and alternaria leaf blight disease severity (Figure 1). Consequently, as disease severity (PDI) increased, the yield dropped. The projected linear regression line has a decreasing slope as well, i.e y=-274 x+242, with a regression coefficient R^2 = 0.83, where "y" denoted predicted grain yield (kg/ha) of linseed genotypes and "x" stood for percent disease index of alternaria leaf blight disease of linseed genotypes (Figure 1).

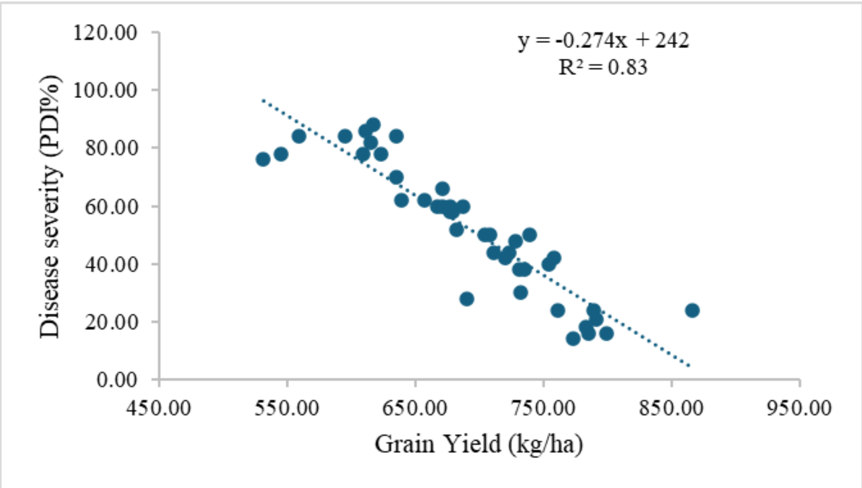

Figure 1. Relationship between grain yield (kg/ha) and alternaria leaf blight disease severity (PDI) of linseed genotypes at ORP, Nawalpur, Sarlahi, Nepal during 2021-2022

The experiment revealed that 19% of the tested genotypes (ACC#(5-ICLI-2001-5), ACC#96-001, ACC#7622, ACC#96-004, TN#04, ACC#(9-ICLI-2001-9), ACC#(1-ICLI-2001-1) and TN#08) exhibited moderately resistance to *alternaria leaf blight*, 31% were moderately susceptible, 26% were susceptible, and 24% were highly susceptible (Table 4).

The experiment demonstrated variability in the resistance levels of linseed genotypes against Alternaria leaf blight, with 19% exhibiting moderate resistance. This result underscores the genetic diversity in linseed for disease resistance, a critical aspect for breeding programs aimed at enhancing crop resilience. Among the moderately resistant genotypes, ACC#(5-ICLI-2001-5), ACC#96-001, ACC#7622, ACC#96-004, TN#04, ACC#(9-ICLI-2001-9),

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ACC#(1-ICLI-2001-1), and TN#08 showed promise as potential donors for resistance traits. Similar findings were reported by Paliwal et al. (2024), who screened linseed genotypes and found a comparable percentage exhibiting moderate resistance to Alternaria blight. These studies collectively suggest that moderate resistance is achievable and can serve as a foundation for breeding resistant varieties.

The moderately resistant genotypes may employ various defense mechanisms, including the production of phytoalexins, the activation of systemic acquired resistance (SAR), and enhanced lignification of cell walls (Vance *et al.,* 1980). Van *et al.* (2001) identified the accumulation of secondary metabolites like phenolics and flavonoids as contributing factors in resistant linseed genotypes, which hinder pathogen proliferation. Additionally, enhanced enzymatic activities, such as peroxidase and polyphenol oxidase, might play a role in reducing disease severity, as suggested by studies on oilseed crops by Thomma *et al.* (1999).
A significant portion of the tested genotypes, 31%, was moderately susceptible, while 26% were susceptible, and 24% highly susceptible. This wide range aligns with findings by Das et al. (2016), who also noted high susceptibility among several linseed genotypes in screening trials. Their study emphasized the role of environmental factors and pathogen aggressiveness in influencing disease expression, further corroborating the variable resistance observed in this study.

Screening for disease resistance in linseed has been pivotal in other research as well. Ram *et al.* (2007) reported similar trends where a small percentage of genotypes exhibited moderate resistance, while the majority ranged from susceptible to highly susceptible. These alignments reinforce the need for continued exploration of genetic resources to identify and utilize resistant germplasm effectively.

he presence of moderately resistant genotypes in this study is encouraging for future breeding efforts. However, the susceptibility observed in over half of the tested genotypes highlights the urgency to integrate molecular tools such as marker-assisted selection (MAS) and genome-wide association studies (GWAS) to identify resistance genes.

Overall, these findings enhance our understanding of linseed's resistance to Alternaria blight, supporting breeding efforts and further exploration of the biochemical and genetic mechanisms behind disease resistance. Future studies should focus on validating these resistant genotypes under diverse environmental conditions and elucidating their molecular responses to Alternaria blight.

CONCLUSION

This study highlights the variability in resistance levels among linseed genotypes against alternaria leaf blight, with 19% demonstrating moderate resistance. Genotypes such as ACC#(5-ICLI-2001-5), ACC#96-001, ACC#7622, ACC#96-004, TN#04, ACC#(9-ICLI-2001-9), ACC#(1-ICLI-2001-1), and TN#08 are identified as potential sources of resistance. The observed resistance may be attributed to mechanisms like phytoalexin production, enzymatic activity, and structural barriers. However, the susceptibility of over half the genotypes underscores the need for targeted breeding strategies and molecular approaches to improve resistance. Most of the genotypes showed moderately resistant reaction and could be used in breeding programs to develop new varieties that are more resistant to alternaria leaf blight disease.

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Authors' Contributions

S Subedi designed the experiment and conducted it in the field. S. Neupane and S.Yadav helped to observe the data in experiments. S Subedi analyzed the data and prepared the whole manuscript. J. Shrestha reviewed initial draft of manuscript, provided critical feedback on the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors have no relevant financial or non-financial interests to disclose.

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