

EVALUATION OF RAPESEED GENOTYPES AGAINST ALTERNARIA BLIGHT UNDER FIELD CONDITIONS IN NAWALPARASI-WEST, NEPAL

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ABSTRACT

Alternaria blight disease causes both yield and quality loss in Brassica crops. A field evaluation of ten rapeseed genotypes against Alternaria blight disease was conducted during November 2018 to February 2019 in Parasi, Nawalparasi-west district. Experiment was laid out in a randomized complete block design with individual plot size of 2.25 m² with three replications. Post-harvest in-vitro seed infection test was done in a completely randomized design with four replications. Disease scoring was done as percentage of leaf area and pod infection on individual 15 sample plants per plot at seven days intervals. Genotype ICT 2001-35 was found moderately resistant based on categorization of mean leaf AUDPC (308.52) and mean pod infection AUDPC values (391.48) with low seed infection (12.50%). Preeti, Bikash and Pragati showed highly susceptible reactions to both leaf blight and pod infection. Therefore, genotype ICT 2001-35 could be used in a varietal improvement program for disease resistance against Alternaria blight.

Keywords: Alternaria blight, AUDPC, genotypes, moderately resistant, rapeseed

INTRODUCTION

Oilseed is one of the important cash crops of Nepal. It is mostly grown after monsoon maize in upland conditions and after early rice in lowland of terai, inner terai and mid-hills (Ghimire *et al.*, 2000). It occupied 258,141 hectares of land with the production of 278,325 metric tonnes and productivity of 1.08 mt/ha in 2019/20 (MoALD, 2021). Among the oilseed crops, rapeseed (*Brassica campestris* var. *toria*) is the dominant oilseed crop that occupies about 85% of the total oilseed area in Nepal (Basnet, 2005). A wide gap exists between the potential yield and the yield realized at the farmer's field due to a number of biotic and abiotic stresses. Among the biotic factors, Alternaria blight disease caused by *Alternaria brassicae* (Berk.) Sacc. has been reported from all the continents of the world causing both yield and quality loss (Sharma and Pandey, 2013). These days, it has become one of the major diseases in Terai and inner Terai regions of Nepal (Shrestha and Chaudhary, 1999). Shrestha *et al.* (2005) reported an average yield loss in the range of 32-57% due to the disease in Nepal. In addition to direct losses in yield, the disease adversely affects seed quality by reducing seed size, seed discolouration and reduction in oil content (Prasad,

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2006). *Alternaria* is a very destructive pathogen causing a widespread destruction in vegetables and other economically important crops (Mamgain *et al.*, 2013). The environmental conditions 80-90% RH, maximum temperature ranging from 18-25 °C, minimum temperature ranging from 10-14 °C and 14-15 hour wetness period daily with dews from 6 pm to 9 am during the month of December and January, favored the disease development of *Alternaria* leaf blight in mustard (Shrestha *et al.*, 2005).

Alternaria blight is the most destructive disease and no well characterized source of resistance is available (Labana *et al.*, 2013). Mamgain *et al.* (2013) reported keeping in view the various health hazards to human beings by chemical control, growing disease resistant varieties is more economical, eco-friendly and safe. Thus, the objective of this study was to assess the level of resistance in different rapeseed genotypes against *Alternaria* blight disease under field conditions of Nawalparasi-west, central inner terai district of Nepal.

METHODOLOGY

FIELD EXPERIMENT

Field experiment was conducted in Ramgram Municipality-4, Nawalparasi-west during November 2018 to February 2019 and in-vitro test for seed after harvest was conducted at plant pathology laboratory of Agriculture and Forestry University, (AFU), Rampur, Chitwan. Field experiment was conducted in a randomized complete block design (RCBD) with 3 replications. Individual plot size was 2.25 m² (1.5 m × 1.5 m) and the area of the research field was 107.38 m². There were six rows of 1.5 m length/plot at a distance of 10 cm apart. The susceptible check variety, "Pragati" was sown in a single row around the whole field. Interblock and interplot spacing were 80 cm and 40 cm, respectively. Ten rapeseed genotypes (Preeti, Bikash, Pragati, Unnati, Uttara Tori, Acc # 9118, Acc # 9109, Local Kalo Tori, Morang Tori 2, and ICT 2001-35) were randomly allocated in each block. Analysis of variance (ANOVA) was used to test differences among the treatments and means separated using Duncan's multiple range test (DMRT) at 5% level of significance.

DISEASE ASSESSMENT

Disease observations were taken from 15 plants selected randomly from six central rows of each plot. The plants were tagged for further scoring. Disease intensity was scored on a 0-5 scale (Sharma and Kolte, 1994) as below.

- 0 = no symptoms
- 1 = 1-10% leaf area covered by spots
- 2 = 11-25% leaf area covered by spots
- 3 = 26-50% leaf area covered by spots
- 4 = 51-75% leaf area covered by spots
- 5 = >75% leaf area covered by spots

The severity on pods was also measured from the sample plants after initiation of pod formation. Disease scoring scale for severity on pod was 0-5 scale (Sapkota *et al.*, 2002) as follows:

- 0 = No symptoms
- 1 = Infection on leaf and started on stem
- 2 = 5% area of stem covered by lesions
- 3 = 6-25% area of stem covered by lesions, initiation of lesion on siliques
- 4 = 50% area of stem covered by lesions and sufficient lesions on siliques
- 5 = >50% area of inflorescence and siliques covered by lesions

The percent disease severity (%) of foliar diseases at each scoring was calculated by using following formula developed by (Sharma and Kolte, 1994):

$$\text{Disease severity (\%)} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of plants observed} \times \text{maximum rating}} \times 100$$

Disease severity was calculated/plant and mean severity was computed/plot. The area under disease progress curve (AUDPC) value was calculated by using the following formula as given by Das *et al.* (1992).

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left[\frac{Y_i + Y_{(i+1)}}{2} \times (t_{(i+1)} - t_i) \right]$$

Where, Y_i = disease severity on the i^{th} date

Y_{i+1} = disease severity on the $i+1^{\text{th}}$ date

N = number of dates on which disease was recorded

Disease severity was measured on leaf and pods separately. Total AUDPC, mean AUDPC and AUDPC per day were also calculated. Based on mean leaf AUDPC values and mean pod AUDPC values obtained from the observation of ten genotypes, a scale of mean leaf and pod AUDPC value was proposed to categorize the genotypes into three resistance levels as below:

Mean leaf AUDPC value	Mean pod AUDPC value	Resistant category	Code
301-350	350-400	Moderately resistant	MR
351-400	401-450	Susceptible	S
>400	>450	Highly susceptible	HS

SEED BORNE INFECTION

Randomly selected 400 seeds of each test genotype were tested for the presence of pathogen. Twenty-five seeds were placed at equidistance per Petri dish with two layers of moistened blotting paper. Seeds plated in the Petri dishes were incubated at $25\pm 1^{\circ}\text{C}$ for seven days. The plates were transferred to a deep freezer at -20°C for 12 hours after 24 hrs of incubation and re-incubated at $25\pm 1^{\circ}\text{C}$ for 6 days. On the 7th day of incubation period the seeds were examined thoroughly under different magnification of stereomicroscope for the growth of *Alternaria brassicae* on them. Seed infection percentage was calculated using the following formula:

$$\text{Seed infection \%} = \frac{\text{Number of infected seeds} \times 100}{\text{Total number of seeds observed}}$$

Saprophytic and pathogenic species were identified based on the size of conidia. Conidia are distinctive; darkly pigmented, oval and both horizontal and vertical internal walls (septa). Conidia having elongated terminal cells ("beaks" or "tails") are generally pathogenic whereas saprophytic *Alternaria* spp. do not have beaks.

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were done by using statistical software R-STAT; correlation analysis was done using MS-Excel 2010.

RESULTS

DISEASE INCIDENCE AND SEVERITY

Symptoms of *Alternaria* blight appeared in all rapeseed genotypes 63 to 70 days after sowing (DAS). Disease incidence at 63 DAS was found highest in Preeti (23.33%) and lowest was seen in Morang Tori-2 (4.56%) which was at par with Local Kalo Tori (8.31%).

Ten rapeseed genotypes varied considerably in *Alternaria* blight disease at 82, 89 and 96 DAS. Disease severity observed at 82 DAS was found highest in Preeti (51.11%) followed by Bikash (50.67%), Pragati (49.78%) and Uttara Tori (48.89%). Minimum leaf severity was observed in ICT 2001-35 (36.44%) which was at par with ACC # 9109 (40.89%) and Local Kalo Tori (40.89%).

Disease severity observed at 89 DAS was found highest in Bikash (72.88%) which was at par with Preeti (66.66%), Pragati (65.77%) and Unnati (65.77%). Minimum leaf severity was found in ICT 2001-35 (52.88%) which was at par with Morang Tori-2 (56.00%), Local Kalo Tori (56.00%) and ACC # 9109 (57.33%), respectively (Table 1).

Disease severity observed at 96 DAS was found highly significant among genotypes with highest being in Bikash (90.22%) which was at par with Preeti (88.44%) and lowest severity was found in ICT 2001-35 (72.00%), which was at par with Morang Tori-2 (76.44%), respectively. Minimum disease severity in leaf is found in genotype ICT 2001-35.

Table 1. Severity of Alternaria blight disease in rapeseed genotypes in Parasi, Nawalparasi-west, Nepal during Nov 2018 to Feb 2019

Genotypes	82 DAS	89 DAS	96 DAS
Preeti	51.11 ^a	66.66 ^{ab}	88.44 ^a
Bikash	50.67 ^a	72.88 ^a	90.22 ^a
Pragati	49.78 ^{ab}	65.77 ^{abc}	88.00 ^a
Unnati	45.33 ^{abc}	65.77 ^{abc}	84.88 ^{ab}
Uttara Tori	48.89 ^{ab}	59.55 ^{bcd}	81.77 ^{ab}
ACC # 9118	43.56 ^{abc}	59.55 ^{bcd}	82.66 ^{ab}
ACC # 9109	40.89 ^{bc}	57.33 ^{bcd}	77.33 ^{bc}
Local Kalo Tori	40.89 ^{bc}	56.00 ^{cd}	82.22 ^{ab}
Morang Tori 2	42.22 ^{abc}	56.00 ^{cd}	76.44 ^{bc}
ICT 2001-35	36.44 ^c	52.88 ^d	72.00 ^c
LSD	8.21	8.78	8.43
p-value	0.01568	0.00332	4.62e-03
Grand mean	44.97	61.24	82.4
SEM(±)	1.25	1.34	1.26
CV (%)	10.64	8.35	5.96

DAS: Days after sowing, CV: Coefficient of variation, LSD: Least significant difference, Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance, SEM(±): Standard error of mean

DISEASE SEVERITY IN POD

Ten rapeseed genotypes varied considerably in pod severity in 89, 96 and 103 DAS. The pod severity observed at 82 DAS was found non-significant. The disease severity at 89 DAS was found highest in Bikash (66.67%) which was at par with Preeti (62.66%),

Pragati (61.33%) and Unnati (59.11%). Lowest pod severity was found in ICT 2001-35 (47.56%) which was at par with ACC # 9118 (51.11%), and Morang Tori-2 (52.89%). Pod severity at 96 and 103 DAS was found highest in Preeti (81.78%, 93.78%) which was at par with Bikash, Pragati and Unnati. Lowest pod severity was found in ICT 2001-35 (63.33%) in 96 DAS and in Morang Tori-2 (83.11%) at 103 DAS.

Table 2. Disease severity in pod of rapeseed genotypes in Parasi, Nawalparasi-west, Nepal during Nov 2018 to Feb 2019

Genotypes	82 DAS	89 DAS	96 DAS	103 DAS
Preeti	39.11	62.66 ^{ab}	81.78 ^a	93.78 ^a
Bikash	35.11	66.67 ^a	75.11 ^{ab}	90.67 ^{abc}
Pragati	32.00	61.33 ^{ab}	73.33 ^{ab}	90.22 ^{abc}
Unnati	40.00	59.11 ^{abc}	74.22 ^{ab}	91.56 ^{ab}
Uttara Tori	38.67	53.33 ^{bc}	73.78 ^{ab}	86.33 ^{bcd}
ACC # 9118	28.44	51.11 ^{bc}	71.11 ^{bc}	90.88 ^{abc}
ACC # 9109	35.56	55.56 ^{abc}	67.56 ^{bc}	90.67 ^{abc}
Local Kalo Tori	30.67	56.44 ^{abc}	68.00 ^{bc}	84.44 ^{cd}
Morang Tori 2	35.11	52.89 ^{bc}	71.84 ^{bc}	83.11 ^d
ICT 2001-35	28.44	47.56 ^c	63.33 ^c	85.33 ^{bcd}
LSD		10.93	8.52	5.88
p-value	NS	0.0468	0.0204	0.0157
Grand mean	34.31	56.67	71.93	88.7
SEM(±)	1.18	1.39	1.15	0.83
CV (%)	17.43	11.24	6.91	3.86

DAS: Days after sowing, CV: Coefficient of variation, LSD: Least significant difference, Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance, SEM(±): Standard error of mean, NS: Non-significant

DISEASE RESISTANCE/SUSCEPTIBILITY OF RAPESEED GENOTYPES

According to mean leaf infection, AUDPC values Preeti, Bikash and Pragati were highly susceptible, Unnati, Uttara Tori, ACC # 9118 were susceptible and ACC # 9109, Local Kalo Tori, Morang Tori-2, and ICT 2001-35 were moderately resistant against *Alternaria* blight disease of rapeseed. Based on the mean pod infection AUDPC values, Preeti, Bikash, Pragati, and Unnati were highly susceptible for pod infection; Uttara Tori, ACC # 9118, ACC # 9109, Local Kalo Tori, and Morang Tori-2 were susceptible

and ICT 2001-35 was found moderately resistant for pod infection. ICT 2001-35 was the only genotype found moderately resistant for both leaf blight and pod infection.

Table 3. Categorization of rapeseed genotypes based on mean AUDPC of leaf blight and mean AUDPC of pod infection

Mean leaf AUDPC value	Genotypes	Mean pod AUDPC value	Genotypes	Resistant category
301-350	ACC # 9109, Local Kalo Tori, Morang Tori 2	350-400	ICT 2001-35	Moderately resistant
351-400	Unnati, Uttara Tori, ACC # 9118	401-450	Uttara Tori, ACC # 9118, ACC #9109, Local Kalo Tori, Morang Tori 2	Susceptible
>400	Preeti, Bikash, Pragati	>450	Preeti, Bikash, Pragati, Unnati	Highly susceptible

THOUSAND SEED WEIGHT OF RAPESEED GENOTYPES

There was a significant difference in thousand seed weight among the genotypes. Highest seed weight was found in genotype Bikash (4.76 g) which was at par with ICT 2001-35 (4.68 g), Pragati (4.63 g), Morang Tori-2 (4.57 g), and Uttara tori (4.50 g). Lowest test weight was found in Preeti (4.11 g) followed by Unnati (4.36 g), Local Kalo Tori (4.38 g).

Table 4. Thousand seed weight of rapeseed genotypes in Parasi, Nawalparasi-west, Nepal during Nov 2018 to Feb 2019

Genotypes	1000 seed wt. (g)
Preeti	4.11 ^d
Bikash	4.76 ^a
Pragati	4.63 ^{abc}
Unnati	4.36 ^{cd}
Uttara Tori	4.50 ^{abc}
ACC # 9118	4.49 ^{abc}
ACC # 9109	4.46 ^{abc}
Local Kalo Tori	4.38 ^{bcd}

Genotypes	1000 seed wt. (g)
Morang Tori 2	4.57 ^{ab}
ICT 2001-35	4.68 ^{ab}
LSD	0.27
p-value	0.005
Grand mean	4.49
SEM(±)	0.04
CV (%)	3.51

CV: Coefficient of variation, LSD: Least significant difference, Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance, SEM (±) represents standard error of mean

CORRELATION BETWEEN THE PARAMETERS OF DISEASE AND THOUSAND SEED WEIGHT

Pearson's correlation coefficients were analysed between different variables. Mean AUDPC on pod showed negative correlation with thousand seed weight (-0.346). Final disease severity on pod showed highly significant positive correlation with mean AUDPC on pod infection (0.636) and significant negative correlation with thousand seed weight (-0.151). Mean AUDPC of leaf spot showed highly significant positive correlation with both final disease severity on pod (0.510) and mean AUDPC on pod infection (0.733).

Final disease severity showed highly significant positive correlation with mean AUDPC of leaf spot (0.767), significant positive correlation with final disease severity on pod (0.329), and highly significant positive correlation with mean AUDPC on pod infection (0.741). Disease incidence showed highly significant positive correlation with final disease severity (0.617), mean AUDPC of leaf spot (0.710), mean AUDPC on pod infection (0.665) and significant positive correlation with final disease severity on pod (0.355).

Disease parameters such as disease incidence, final disease severity, mean AUDPC of leaf spot, final disease severity on pod and mean AUDPC on pod showed significant negative correlation with thousand seed weight.

Table 5. Pearson's correlation between different disease parameters and thousand seed weight, Parasi, Nawalparasi-west, Nepal during Nov 2018 to Feb 2019

	Disease incidence	Final disease severity (%)	Mean AUDPC of leaf spot	Final disease severity on pod	Mean AUDPC on pod infection	Thousand seed weight
Disease incidence	1	.617**	.710**	0.355	.665**	-0.158
Final disease		1	.767**	0.329	.741**	-0.159

Severity (%)				
Mean AUDPC of leaf spot	1	.510**	.733**	-0.042
Final disease Severity on pod		1	.636**	-0.151
Mean AUDPC on pod			1	-0.346
Thousand seed weight				1

** : Correlation is significant at the 0.01 level (2-tailed)

* : Correlation is significant at the 0.05 level (2-tailed)

SEED INFECTION

Seeds of all the varieties were infected with *Alternaria* spp., however, the level of infection differed significantly ($p \leq 0.05$). The highest seed infection was found in Preeti (45.25%) which was at par with Unnati (40.75%), Bikash (39.75%) and Pragati (39.25%), and lowest infection was recorded in Local Kalo Tori (12.00%) followed by ICT 2001-35 (12.50%) and Morang Tori-2 (14.75%).

Table 6. Seed infection by *Alternaria brassicae* in ten rapeseed genotypes after harvest, in Parasi, Nawalparasi-west, Nepal during Nov 2018 to Feb 2019

Genotypes	Seed infestation (%)
Preeti	45.25 ^a
Bikash	39.75 ^a
Pragati	39.25 ^a
Unnati	40.75 ^a
Uttara Tori	22.25 ^b
Acc # 9118	17.75 ^b
Acc # 9109	17.75 ^b
Local Kalo Tori	12.00 ^b
Morang Tori 2	14.75 ^b
ICT 200-35	12.50 ^b
LSD	11.95
p-value	4.34e-07
Grand Mean	26.2
SEm(±)	2.33
CV(%)	31.6

CV: Coefficient of variation, LSD: Least significant difference, Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance, SEm (±): Standard error of mean

REGRESSION ANALYSIS

There were significant positive linear correlations between mean AUDPC of leaf and seed infection, and between mean AUDPC of pod infection and seed infection percent (Figure 1). According to linear regression equation, it could be said that if there is an unit increase in mean AUDPC of leaf, seed infection percent would have been increased by 0.336 times and if there is an unit increase in mean AUDPC of pod infection, seed infection percent would have been increased by 0.422 times. According to the coefficient of determination, about 83.4% variation in seed infection percent was due to mean AUDPC of leaf and about 85.4% variation in seed infection percent was due to mean AUDPC of pod infection and remaining portion was due to other factors. It is reasonable to conclude that there is positive correlation between mean AUDPC of leaf with seed infection, and mean AUDPC of pod infection with seed infection percent. Increase in mean AUDPC of leaf and mean AUDPC of pod infection significantly increases the seed infection percent.

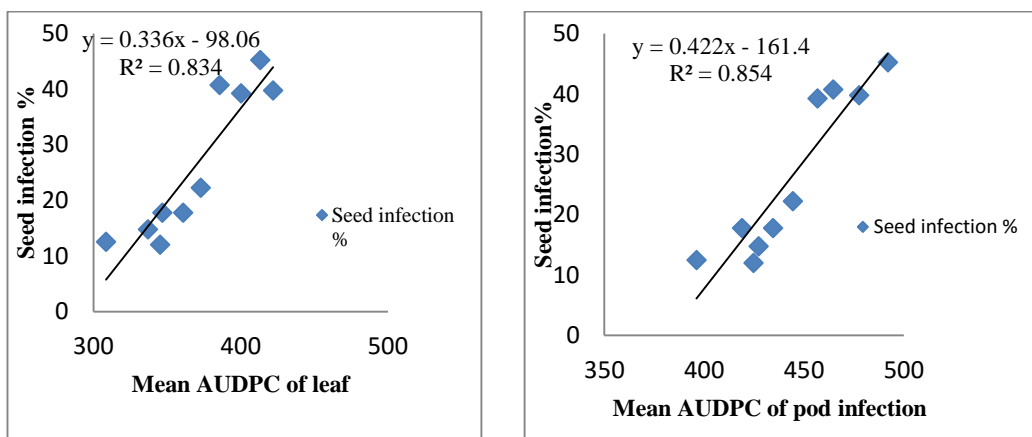


Figure 1. Regression showing the relationship between mean AUDPC of leaf infection and mean AUDPC of pod infection with seed infection in rapeseed genotypes

DISCUSSIONS

Alternaria blight severity in leaf ranged from 72% to 90.22% whereas pod severity ranged from 63.33 to 81.78% at 96 days after sowing. ACC # 9109, Local Kalo Tori, and Morang Tori 2 showed moderately resistant, Unnati, Uttar tori and ACC # 9118 showed susceptible and Preeti, Bikash, Pragati showed highly susceptible reaction to leaf blight whereas Morang Tori 2, Local Kalo Tori, ACC # 9109, ACC # 9118, and Uttara tori showed susceptible and Preeti, Bikash, Pragati, and Unnati showed highly susceptible to pod infection. Only one genotype ICT 2001-35 showed moderately resistant reaction to both leaf blight and pod infection. None of the genotypes was found disease free or resistant. Singh *et al.* (2018) found seven genotypes resistant,

15 genotypes moderately resistant and rest of the genotypes susceptible and highly susceptible but none was disease free among 200 tested genotypes. Talukdar and Das (2017) also have reported four moderately resistant, nine susceptible, 16 moderately susceptible and one highly susceptible but none was highly resistant among 30 tested genotypes against *Alternaria* blight.

There was significant positive correlation between disease parameters such as final disease severity to mean AUDPC on pod infection but negative correlation between disease parameters and thousand seed weight. This might be due to the fact that blighting of leaves reduced photosynthetic area and pod infection resulting in weight and quality loss of rapeseed. Seed infection by *Alternaria* species caused shriveling of seeds, which reduced thousand seed weight and seed yield (Sapkota *et al.*, 2002).

CONCLUSIONS

The findings revealed that among ten genotypes tested, Preeti, Bikash and Pragati showed highly susceptible reactions to both leaf blight and pod infection, ICT 2001-35 was moderately resistant to both leaf blight and pod infection but none was resistant. Seed infection percentage was found in the range of 12% to 45.25% in different genotypes. Higher seed infection in Preeti (45.25%) was because of higher disease incidence, higher disease severity, higher mean AUDPC values of leaf and pod infection. The genotype ICT 2001-35 was found moderately resistant to *Alternaria* leaf spot and pod blight with less disease severity and less post-harvest seed infection. So, it could be used in a varietal improvement program for disease resistance against *Alternaria* leaf blight.

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