# Noble indices for degree of host plant resistance against Alternaria blight in Rapeseed-mustard

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**ABSTRACT: Experiments were conducted at Genetics and Plant Breeding Research Farm of N. D. University of Agriculture and Technology, Kumarganj, Faizabad (U.P.) to evaluate 15 promising genotypes of rapeseed-mustard against Alternaria blight caused by *Alternaria brassicae* (Berk) Sacc. And *A. brassicicola* (Schw) Wiltshire. It is the most destructive and major disease problem under eastern Uttar Pradesh condition causing both the quantitative and qualitative losses. The evaluation criteria for host resistance against the disease considered were the number and size of spot, number of conidia per spot on both the vegetative (leaf) and reproductive (pod) parts were regularly recorded at periodical intervals starting from the disease appearance till the physiological maturity of each and every genotypes. The indices which were considered for in built resistance were delayed appearance of visible symptoms, minimum number of spot and their size, number of conidia per spot as well as the minimum leaf defoliation. The disease appeared first in genotype T-9 (44 DAS) which was delayed in GSL-5 (53 DAS). The number of spot/10cm$^2$, size, sporulation capacity in terms of conidia per spot varied invariably among the genotypes and significantly in some cases.**

**The appearance of disease symptoms in the form of blighted spots varied invariably (44 to 53 DAS) in different genotypes. The delayed appearance of disease symptoms (53 and 52 DAS), lower number of spot / 10cm$^2$ on leaf (3.07 and 3.65), pods (3.74 and 4.14), smallest spot size on leaf (3.54 and 3.05 cm), pods (1.22 and 1.27 cm), number of conidia per spot on leaf (1230 and 1630), pods (130 and 150) were visualised in genotype GSL-5 and Pusa Aditya,respectively indicating a certain pattern and may be considered as attributes of disease resistance. Further, this fact was strengthened with minimum leaf defoliation (32.03 and 35.55%), PDI on leaf (12.35 and 14.16), pods (7.73 and 7.93) and ultimately the AUDPC calculated on leaf (486.70 and 551.90) and pods (282.85 and 291.05). Accordingly both the genotypes were grouped in moderately resistant (MR) category. The correlation coefficient (r) among parameters attributing to resistance behaviour worked out proved helpful in drawing the conclusion. Because very limited source of durable resistance are available, it may be suggested to exploit the two genotypes *i.e.*GSL-5 and Pusa Aditya for breeding programme against Alternaria blight.**

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**Key words**: Genotypes, Alternaria blight, PDI, disease progression, AUDPC



## Introduction

India is one of the largest rapeseed-mustard growing countries in the world, occupying the third position in area and production after China and Canada sharing 12% of world's total production. At global level, rapeseed-mustard is cultivated on 36.15 m ha with production of 71.09 MT (Anonymous, 2016[a]). Rapeseed-mustard is the second most important oilseed crop after groundnut and accounts for nearly 30.7% of the total oilseed production in the country.

In spite of higher yield potential, diseases are major constraints, of which Alternaria blight caused by *Alternaria brassicae* (Berk.) Sacc. is one of the most severe and yield destabilizing factor. It attacks stems, leaves and pods and causes an average yield loss of 35-40% (Kumar *et al.* 2014) whereas, the reduction 27.24% in seed yield and 5.98% in 1000 grain weight due to the disease were assessed by Mahapatra and Das (2017). In addition to the direct loss of yield, the disease adversely affects the seed quality by reducing seed size, seed colour, germination, vigour index and oil content (Kaushik*et al.*, 1984). Use of fungicides and their indiscriminate use not only pollute the environment but has an adverse residual effect on soil health and fertility. In spite of that, there is unawareness among farmers regarding application of appropriate chemicals, doses and schedule. Farmers are not adopting proper cultural practices helpful to combat the disease. The information available on the sources of resistance is scanty. Therefore, the research on identification of resistant sources in rapeseed-mustard to Alternaria blight has got increasing attention over past few years. Overall the goal of the present study was to determine different Alternaria blight resistance contributing and assessing attributes.

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## MATERIALS AND METHODS:

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Field experiments were conducted at Genetics and Plant Breeding Research Farm of N. D. University of Agriculture and Technology, Kumarganj ($26^0$ 47'N, $82^0$ 12'E, 113msl), Faizabad, Uttar Pradesh, India, during 2015-16 and 2016-17 cropping season. Sowing was done in first fort night of November during both the years and recommended agronomic practices were followed. Fifteen genotypes of rapeseed-mustard were grown in a randomized block design, having plot size of 4.0 m x 3.0 m and spacing 30 cm x 15 cm row to row and plant to plant, respectively.

The crop was regularly monitored for the first appearance of disease. The per cent disease severity in each treatment was recorded using 0-9 scale as suggested in proceedings of All India Coordinated Research Project on Rapeseed-Mustard Pathology, Planning and Review Session 2015-16 (Anonymous, 2016). Observations were noted at 10 days interval on lower, middle and upper leaves and finally on pods of randomly selected 10 plants in each treatment and replication. Observations were taken at every 10 days interval.

The number of spots were counted per 10 $cm^2$ leaf area on different tagged leaves with the help of a glass slide, on which 5 x 2 $cm^2$ area was marked.Observations were taken randomly at four places per leaf lamina on upper surface of the leaf, starting from lower most leaf to the uppermost fully developed leaves. This method of counting of spots was followed

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in all the successive observations. Average number of spot was calculated. Alternaria blight spots also counted on siliquae one week prior to maturity of the plant. A total of fifty siliquae @ 5 siliquae per plant per genotype were observed and average number of spots per siliqua was calculated.

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Randomly ten plants were selected in each genotype for measuring the spot size. From each plant five leaves were randomly selected on which diameter of randomly selected spots were measured in millimetre. Average size of leaf spot in each genotype was calculated. Five largest spot per infected siliqua of the selected plants were measured and average was calculated on the basis of fifty spots per genotype.

The spore production in different genotypes at different intervals on spots of Alternaria blight, the affected leaves were thoroughly washed in running tap water and the lesion of similar size were taken at different intervals and separated by cork borer (8mm). These lesions were surface sterilised with 0.1% mercuric chloride and further washed repeatedly in sterilised distilled water. Sporulation was observed by suspending sporulated lesions in vials containing a mixture of distilled water + lacto phenol in the ratio of 9:1. These lesions were then shaken vigorously and scrapped with the help of camel hair brush. The conidia were counted with the help of a haemocytometer. Then the value was multiplied by $10^3$ (Kumar and Kolte, 2006)

The infected pods of above genotypes were collected from the field at different intervals and thoroughly washed in running tap water. The pods were cut in 6 mm pieces containing single spot. Fifteen such surface sterilized pieces were incubated in petri plates in a moist chamber for 48 hr at room temperature with alternating 12 hr light and 12 hr dark periods. Conidia were counted as per method described above.

PDI was calculated by selecting five leaves per plant on ten plants from each genotype on a random basis in each replication. Observations were taken at 10 days intervals from first appearance of disease to till highest disease severity and average PDI was calculated. The blighting was assessed as per cent leaf area covered. The per cent disease intensity was calculated by using the following formula:

$$\text{Per cent disease intensity (\%)} = \frac{\text{Sum of numerical ratings} \times 100}{\text{Number of leaves examined} \times \text{Maximum grade}}$$

Area under disease progress curve (AUDPC) was calculated on the basis of per cent disease severity for each genotype by using the formula as given below on the basis of average pooled data recorded during both the years of experimentations.

$$AUDPC = \sum_{i=1}^{n}[(Y_{i+1} + Y_i) \times 0.5\ (T_{i+1} + T_i)]$$

Where

$Y_i$= Alternaria blight severity (%) at the I$^{st}$ observation, $T_i$= Time (days) of the I$^{st}$ observation, n= Total number of observations

Observations on leaf defoliation were taken up to entire growth period of plants. In each observation, leaves were counted from basal to top. Average was taken based on ten plants for each genotype in each replication and per cent leaf defoliation was calculated as follows:

$$\text{Leaf defoliation (\%)} = \frac{\text{Total number of leaves defoliated}}{\text{Total number of leaves on main axis}} \times 100$$

The yields of net area of each plot without border rows were obtained. The obtained yield of each genotype was calculated in q/ha. The statistical analysis for each and every character was done on the basis of average of pooled figures of both the years of experimentations and there conversion in transformed value wherever necessary.

## RESULTS AND DISCUSSION:

Significant differences among genotypes and time intervals were observed for all the components of disease resistance *viz*., number and size of spots, number of conidia per spot and per cent disease intensity.The disease first appeared on the genotype Parwati and T-9 (44 DAS) followed by KOS-1, PS-66 and Benoy (45 DAS) during both the years as presented Table 1.

The minimum number of spots on leaves/10cm$^2$ was recorded on genotype GSL-5 (3.07) followed by genotypes Pusa aditya (3.65) and Anuradha (4.47), respectively. The significantly high number of spot /10 cm$^2$ was recorded on genotype Parwati (6.87) followed by PC-5(6.67) and KOS-1 as well as Benoy (6.47), respectively. Likewise, the least number of spots on pod recorded on genotype GSL-5 (3.74) followed by Pusa Aditya (4.14), respectively. The maximum number of spots on pods was recorded on the cultivar Patan sarson-66 (16.74) followed by TMLC-2 (13.34), Giriraj (12.94) and Pusa mustard-25 (10.34) respectively.

The minimum size of spot on leaf was recorded on genotype GSL-5 (3.05 mm) followed by Pusa aditya (3.54) and TMLC-2 (4.54 mm). Patan sarson-66 showed maximum size of spot (9.26 mm) followed by Pusa mustard-27 (8.66 mm) and Giriraj (8.46 mm), respectively, and these were at par with each other. On the pods, size of spots (mm) ranged from 1.22 to 6.62. Minimum size of spots on pod was observed on genotype GSL-5 (1.22 mm) followed by Pusa aditya (1.27 mm) and KOS-1 (1.77mm), respectively. The maximum

size of spots on pod was recorded on cultivar Parwati (6.67 mm) followed by Pusa gold-45 (6.07 mm).

Similar trend was recorded in case of sporulation or the number of conidia per leaf spot in different genotypes. The minimum number of conidia/leaf spot and per pod was recorded in the spot of genotype GSL-5 (1230 and 130) followed by Pusa aditya (1630 and 150), respectively. The maximum number of conidia per spot was recorded on cultivar Patan sarson-66 (5200) followed by TMLC-2 (5030), T-9 (4900) and Parwathi (4400). The maximum number of conidia per spot on pods was recorded on cultivar Patan sarson-66 (570) followed by cultivar Pusa gold and Benoy (460) and TMLC-2 (430), respectively.

Significantly lower per cent disease severity on leaves was recorded on cultivar GSL-5 (12.35%) followed by Pusa aditya (14.16%). The maximum per cent disease severity (PDI) on leaves was observed on genotype TMLC-2 (48.32%) followed PS-66 (41.98%). The minimum per cent disease severity on pod was recorded on cultivar GSL-5 (7.73%) followed by the Pusa Aditya (7.93%), respectively. The maximum per cent disease severity on pod was recorded on cultivar PS-66 (43.90%). Genotypes Parwati, PS-66, Pusa gold-45, Benoy showed susceptibility and varieties GSL-5, Pusa aditya, Giriraj, RH-406 were recorded resistant.

The minimum AUDPC was recorded on leaf and pod of GSL-5 (486.70 and 282.85) followed by Pusa aditya (551.90 and 291.05). The maximum AUDPC was observed on cultivar TMLC-2 (1962.70) followed by PS-66 (1687.00), and T-9 (1578.95), respectively.

The present findings collaborates with findings of Kumar and Kolte (2001) who reported progression of Alternaria blight disease (*Alternaria brassicae*) in nine genotypes of mustard in relation to different resistance components, *viz.*, number of spots, size of spot, sporulation, disease index, apparent infection rate, area under disease progress curve (AUDPC), leaf defoliation and their effect on yield potential were studied under field conditions. Similar observations on all these indices contributing for resistance were also considered by Kumar and Kolte (2006) but no correlation studies between these traits were worked out as done during the present investigation. All the components of resistance were correlated significantly positive with each other. The yield potential was correlated significantly negative with all the components except with leaf defoliation. The genotypes PR-8988 and PR-9024 showed significantly reduced number of spots (4.36 - 15.89), smaller

size of spot (2.12 - 6.17 mm), lower sporulation (0.30 - 1.84 x $10^3$ conidia), lower disease index (36.51-42.20%), reduced apparent infection rate (r = 0.047-0.080), lesser values of AUDPC (45.35-126.70) on leaf and pod, respectively along with reduced leaf defoliation (38.40-44.40%) in comparison to national susceptible genotype Varuna.

Correlations showed that all the components were highly significant and positively correlated with each other except sporulation which has negative correlation with yield. The highest value of correlation was recorded between disease index and AUDPC (R= 0.998) followed by sporulation and AUDPC (R= 0.888), where as lowest value of correlation was recorded between number of spot and sporulation (R= 0.282). It shows disease index is the most determinant factor for partial resistance that greatly influences the development and progression of epidemic and negatively correlated with yield.

Partial resistance includes phenomenon such as field resistance (Vander Plank, 1968) and rate reducing resistance (Parlevliet, 1979). Slow blighting may be defined as reduction in infection of the plant by the blight pathogen, the late appearance of blight symptoms in the life cycle of the host and reported growth and development of the pathogen. During present investigation, out of fifteen genotypes examined for blight resistance, GSL-5 and Pusa Aditya were moderately resistant and exhibited good level of partial resistance. Other thirteen genotypes KOS-1, PC-5, RH-406, TMLC-2, Parwati, Pusa mustard-25, Anuradha, Giriraj, T-9, Patan sarson-66, Pusa mustard-27, Pusa gold-45, Benoy, were susceptible based on mean of the disease severity (PDI). Wilcoxson (1986) evaluated slow rusting in cereals based on beak severity. Kumar (2000), Kumar and Kolte (2001) also assessed the field resistance to Alternaria blight (*Alternaria brassicae*) in nine genotypes of *Brassica juncea* under field condition and reported three genotypes *viz.*, PR-8988, PR-9024 and Kranti as partial resistance with lower per cent blight cover, apparent infection rate and AUDPC values. Kumar *et al.* (2014[a]) considered 14 genotypes of rapeseed-mustard and reported that no single genotype was found resistant against various pathotypes of pathogen. During present investigations, the lower AUDPC values clearly separated the rapeseed-mustard genotypes with high level of partial resistance. AUDPC has been used successfully to evaluate the progress of disease rate on different crops also (Kumar and Kolte, 2001, Kumar, 2008). Accordingly, it is evident from the data that genotypes with high level of partial resistance

have good yield potential than susceptible ones which may be attributed with the cumulative effect of less number, reduced size of spots, lower sporulation and leaf defoliation, which supported the findings of earlier worker of mustard (Kumar and Kolte, 2001; Kumar and Kolte, 2007; Kumar 2008; Mamgain et al. 2014 and Talukdar and Das, 2015) as larger lesion developed on susceptible rapeseed-mustard crops than resistant *Brassica* spp.

Different workers from different places have also reported that defoliation of leaves can be used as a parameter of resistance in *Brassica* spp. (Kumar and Kolte 2001, 2007; Kumar 2008 and Mamgain *et al.* 2014). The minimum defoliation of older leaves in cultivar GSL-5 (32.03), Patan sarson-66 (33.55) and KOS-1 (33.87) as compared to maximum in Anuradha (61.55, Parwati (55.05), Benoy (52.99) and Pusa Gold-55 (51.28) indicated as negative trait for selection contributing for resistance.

The maximum seed yield was recorded in cultivar Pusa mustard-25 (3200.40 kg/ha) followed by GSL-5 (2944.85 kg/ha). Others were at par in respect of seed yield. The minimum yield was recorded on cultivar TMLC-2 (815.95 kg/ha) followed by PS-66 (955.96 kg/ha).

The highest value in terms of disease index correlated with AUDPC indicated that it is the most determinant factor and can be utilized as a potent tool during the assessment of disease resistance in varietal evaluation programme especially in case of Alternaria blight of Rapeseed-mustard.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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**Table 1. Evaluation of slow blighting components on the progress of Alternaria blight on Rapeseed-mustard under field condition**

| S. No. | Genotypes | Appearance of disease (DAS) | No. of spots/10 $cm^2$ | | Size of spot (mm) | | Sporulation (conidia/spot) | |
|---|---|---|---|---|---|---|---|---|
| | | | Leaf | Pod | Leaf | Pod | Leaf | Pod |
| **1** | GSL-5 | 53 | 3.07 | 3.74 | 3.54 | 1.22 | 1230 | 130 |
| **2** | KOS-1 | 45 | 6.47 | 5.74 | 6.40 | 1.77 | 3230 | 200 |
| **3** | PC-5 | 50 | 6.67 | 5.34 | 6.66 | 4.47 | 2760 | 170 |
| **4** | RH-406 | 49 | 5.47 | 4.94 | 8.26 | 3.47 | 2160 | 170 |
| **5** | TMLC-2 | 46 | 5.50 | 13.34 | 4.23 | 3.45 | 5030 | 430 |
| **6** | Pusa Aditya | 52 | 3.65 | 4.14 | 3.05 | 1.27 | 1630 | 150 |
| **7** | Parwati | 44 | 6.87 | 6.14 | 7.86 | 6.67 | 4400 | 200 |
| **8** | Pusa mustard-25 | 47 | 6.07 | 10.34 | 6.86 | 4.07 | 4230 | 250 |
| **9** | Anuradha | 49 | 4.47 | 6.94 | 7.86 | 4.87 | 3460 | 210 |
| **10** | Giriraj | 50 | 4.60 | 12.94 | 8.46 | 4.63 | 2300 | 190 |
| **11** | T-9 | 44 | 5.47 | 6.34 | 7.46 | 4.37 | 4900 | 280 |
| **12** | Patan sarson-66 | 45 | 6.27 | 16.74 | 9.26 | 4.27 | 5200 | 570 |
| **13** | Pusa mustard-27 | 48 | 5.47 | 7.14 | 8.70 | 4.67 | 3930 | 410 |
| **14** | Pusa gold-45 | 47 | 6.27 | 5.94 | 6.66 | 6.07 | 4330 | 460 |
| **15** | Benoy | 45 | 6.47 | 7.94 | 7.46 | 4.05 | 4000 | 460 |
| | **C.D at 5%** | | **1.26** | **1.43** | **1.37** | **0.78** | **112.00** | **34.80** |
| | **SEm±** | | **0.21** | **0.56** | **0.32** | **0.27** | **25.00** | **3.00** |
| | **C.V** | | **5.94** | **5.93** | **5.64** | **4.43** | **6.43** | **6.67** |

**Table 2.Evaluation of slow blighting components on the progression of Alternaria blight on rapeseed-mustard under field condition**

| S. No. | Genotypes | Leaf defoliation (%) | Per cent disease intensity | | AUDPC | | Host reaction group | Yield (kg ha$^{-1}$) | Test weight (g) |
|---|---|---|---|---|---|---|---|---|---|
| | | | Leaf | Pod | Leaf | Pod | | | |
| **1** | GSL-5 | 32.03 | 12.35 (20.53) | 7.73 (16.11) | 486.70 | 282.85 | MR | 2944.85 | 3.38 |
| **2** | KOS-1 | 33.87 | 33.62 (35.43) | 32.58 (34.82) | 1361.35 | 1263.30 | S | 1133.74 | 2.79 |
| **3** | PC-5 | 37.36 | 29.67 (33.02) | 37.80 (37.94) | 1176.00 | 1493.40 | S | 1222.63 | 2.96 |
| **4** | RH-406 | 43.07 | 26.37 (30.92) | 29.56 (32.96) | 1037.35 | 1156.50 | S | 1500.40 | 4.59 |
| **5** | TMLC-2 | 37.99 | 48.32 (44.03) | 39.64 (39.00) | 1962.70 | 1545.90 | S | 815.95 | 3.22 |
| **6** | Pusa aditya | 33.86 | 14.16 (22.14) | 7.93 (16.32) | 551.90 | 291.05 | MR | 2667.05 | 3.18 |
| **7** | Parwati | 55.05 | 37.41(37.70) | 40.38 (39.47) | 1475.25 | 1608.50 | S | 1200.09 | 3.33 |
| **8** | Pusa mustard-25 | 43.80 | 38.87 (38.59) | 22.20 (28.11) | 1577.55 | 813.30 | S | 3200.40 | 3.78 |
| **9** | Anuradha | 61.51 | 38.14 (38.12) | 38.50 (38.35) | 1492.85 | 1528.10 | S | 1655.96 | 2.90 |
| **10** | Giriraj | 50.22 | 26.20 (20.79) | 8.25 (16.64) | 1030.10 | 308.60 | S | 1622.63 | 4.90 |
| **11** | T-9 | 49.19 | 39.76 (39.11) | 36.86 (37.41) | 1578.95 | 1430.30 | S | 1889.29 | 4.82 |
| **12** | Patan sarson-66 | 33.55 | 41.98 (40.40) | 43.90 (41.50) | 1687.00 | 1737.00 | S | 955.96 | 3.23 |
| **13** | Pusa mustard-27 | 38.68 | 30.11 (33.27) | 30.56 (33.58) | 1202.05 | 1206.30 | S | 2089.29 | 3.36 |
| **14** | Pusa gold-45 | 51.28 | 37.92 (38.00) | 40.40 (39.47) | 1500.30 | 1593.80 | S | 1222.63 | 5.36 |
| **15** | Benoy | 52.99 | 32.72 (34.88) | 39.94 (39.17) | 1280.35 | 1584.50 | S | 1133.74 | 2.74 |
| | **C.D at 5%** | **3.40** | **11.24** | **9.46** | | | | **221.69** | |
| | **SEm±** | **0.02** | **7.92** | **8.72** | | | | **121.17** | |
| | **C.V** | **5.05** | **13.81** | **13.69** | | | | **8.06** | |

Figures in parenthesis are angular transformed value

**Table 3. Correlation coefficients (r) among various components of partial resistance of Alternaria blight and yield assessment in rapeseed-mustard varieties under field condition**

| Disease components and yield | No. of spots | Size of spot | Disease index | Leaf defoliation | Sporulation | AUDPC | Yield |
|---|---|---|---|---|---|---|---|
| No. of spots | 1.000 | 0.313 | 0.452* | 0.050 | 0.282 | 0.446* | -0.339 |
| Size of spot | | 1.000 | 0.557** | 0.372 | 0.577** | 0.552** | -0.434* |
| Disease index | | | 1.000 | 0.491** | 0.882** | 0.998** | -0.598** |
| Leaf defoliation | | | | 1.000 | 0.367 | 0.464* | -0.271 |
| Sporulation | | | | | 1.000 | 0.888** | -0.505** |
| AUDPC | | | | | | 1.000 | -0.593** |
| Infection rate | | | | | | | -0.566** |
| Yield | | | | | | | 1.000 |

*Significant at 5% ** Significant at 1%