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ASSESSMENT OF GENETIC VARIABILITY IN MUSTARD AGAINST ALTERNARIA LEAF SPOT DISEASE THROUGH NATURAL FIELD CONDITIONS

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A B S T R A C T

The research was conducted for the source of resistance against Alternaria leaf spot disease of brassica. In the current research, ten varieties/ advanced lines were evaluated in 2016-17 and 2017-18 against this disease using Randomized Complete Block Design (RCBD) at the experimental research area of Oilseeds Research Institute, Ayub Agricultural Research Institute Faisalabad. During 2016, three advanced lines (RBN-13017, RBN-13022 and RBN-13029) exhibited moderately resistant response with 22.33%, 18.42% and 13.76% disease incidence respectively under rating 2 whereas two advanced lines namely KN-294 (34.56%) and 14CBN009 (42.99%) expressed a moderately susceptible response (rating 3). Similarly, four advanced lines/ varieties showed susceptible response with (66.54%), (54.28%), (64.77%) and (60.12) percent disease incidence while KJ-159 was used as spreader that expressed highly susceptible response with 92.14 % disease incidence. During 2017-18 four varieties/ advanced lines namely KN-279, 14CBN001, Faisal Canola and Rohi Sarson exhibited susceptible response with (68.84%), (56.38%), (66.97%) and (58.22) percent disease incidence (rating 4) whereas two advanced lines such as KN-294 (31.86%) and 14CBN009 (40.09%) showed moderately susceptible response (rating 3). Likewise, three advanced lines exhibited moderately resistant response with 24.63%, 19.82% and 12.06% disease incidence respectively under rating 2. It was concluded that source of resistance is the most economical management strategy for the farmers against Alternaria Leaf Spot Disease of brassica.

Keywords: *Brassica napus*, *Alternaria brassicae*, source of resistance, filed conditions.

INTRODUCTION

Oilseed crops plays an important role in Pakistan's economy. Mustard (*Brassica napus*) is the most pivotal and largest contributor of oil in Pakistan (Ali *et al.,* 2016). It is highly nutritious, it contains protein (43.6g/kg) and a complete profile of amino acids that includes methionine, cysteine, lysine as well as glucosinolates and erucic acid (Sana *et al.,* 2003). In the world, the estimated area under mustard cultivation is 33.1 million hectares with overall production of 60.7 million tons with average yield of 1832 kg/ha respectively (Singh *et al.,* 2015). The total production of Brassica was 14.7 million tons during 2015-16 in China (Hu *et al.,* 2017) whereas in Pakistan oilseeds are cultivated on an area of 190.3 Thousand ha having total production 162.2 thousand tons (Mahmood *et al.,* 2012).

Rapeseed mustard is threatened by different pathogens like fungal, bacterial and viruses but *Alternaria brassicae* is the most destructive pathogen in mustard cultivating areas of the world (Ali *et al.,* 2016). Pathogen inhibits the seed germination, deteriorate the quality and quantity of oil (Meena *et al.,* 2016). The sever attack enhances the seed

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infection, diminished the pod length, reduces the no. of seed per pod as well as decreases the thousand seed weight and oil contents. *Alternaria brassicae* causes the losses of 25 to 75% in various species of mustard grown in different geographical regions of the world (Singh *et al.,* 2015).

In genus *Alternaria,* polymorphous conidia are formed in single, short or in long chains with longitudinal and oblique septations or cross walls. The spores of polyphagus fungi are present in atmosphere as well as in the soil. The sexual stage (teleomorph) is found in a few species that are placed in genus Pleospora of class Loculoascomycetes (under Sub-division: Ascomycotina). In class Loculoascomycetes muriform ascospores (having sleeper shaped structure) are produced in bitunicate asci (Verma and Saharan, 1994; Singh *et al.,* 2015).

Alternaria leaf spot disease caused by a fungal pathogen i.e *Alternaria brassicae* is considered as most destructive disease in the absence of resistant rapeseed mustard host plants (Ghose *et al.,*2008). Numerous conventional (fungicides and fertilizers application, soil dressing, seed treatments, tillage operations etc.) and non-conventional (plant activators, biocontrol agents, plant extracts, ionic contents comparisons through biochemical analysis etc.) approaches are used for the management of Alternaria leaf spot disease (Bhatt *et al*., 2009). The judicious application of hazardous fungicides, pesticides and fertilizers is not only contaminating the plants but also deteriorating the quality of oil. Therefore, it is need of the hour to find out the resistant sources from the existing rapeseed mustards germplasms at Oilseeds Research Institute, Faisalabad against Alternaria leaf spot disease under field conditions for durable/ horizontal/ field resistance through eco-friendly approaches (Bisht *et al.,* 2015). Moreover, resistant rapeseed mustered germplasms is an adequate, eco-friendly and promising approach to diminish the severity of disease and consequently enhance crop yield, seed production without perilous chemicals and better oil quality with imperative nutrients (Singh *et al.,* 2015). For this purpose efforts were carried out to assess rapeseed mustard germplasm against Alternaria leaf spot disease under natural field conditions.

Aim of current research: Current research was planned to find the existing rapeseed mustard germplasm/ advanced lines under natural field conditions against Alternaria Leaf spot disease caused by *A. brassicae* for the source of resistance.

MATERIALS AND METHODS

During 2016-17 and 2017-18, ten varieties/ advanced lines of rapeseed germplasm viz. RBN-13017, RBN-13029, RBN-13022, KN-279, 14CBN001, KN-294, 14CBN009, Faisal Canola, Rohi Sarson and KJ-159 (Spreader) were collected from Oilseeds Research Station, Ayub Agricultural Research Institute, Faisalabad. Two to four seeds were planted through seed planter at specifically adjusted row length i.e. 11.66 ft or 350 cm by maintaining P×P and R×R distance of 45 cm and 4 cm respectively. The Statistical Randomized Complete Block Design by three replications was adopted for this experiment. After, 20 days of germination, small seedlings were pulled manually to keep the plants in good health as well as to diminish nutrients competition for growth and development. The plants were observed daily for demand based Horticultural and Agronomic field operations namely weed eradication, timely fertilizer application, proper irrigation availability and hoeing. Similarly, the plants were keenly observed for disease appearance to acquire the ultimate or consequent objective of the current research.

DATA RECORDING AND STATISTICAL ANALYSIS

The Disease Incidence (%) data was recorded with seven days interval after the disease symptoms appeared, till harvesting of research trial through following disease incidence formula (reference…) and disease rating scale of Hussain and Thakur (1963). The complete statistical analysis was performed by the use of SAS/STAT 8.1 statistical software (SAS Institute, 1990) whereas the means were separated by using the Fisher's least significant difference (LSD) test by considering P = 0.05% probability level (Steel *et al.,* 1997).

$$\text{Disease Incidence} = \frac{\text{No. of Infected Plants}}{\text{Total No. of observed plants}} \times 100$$

Table 1. Disease rating scale for recording Alternaria leaf spot on *Brassica napus* advanced line/varieties

Rating Scale	% Leave area affected reaction	Reaction	Symbol
0	0	Immune	I
1	1-10	Resistant	R
2	10-25	Moderately Resistant	MR
3	25-50	Moderately Susceptible	MS
4	50-75	Susceptible	S
5	75-100	Highly Susceptible	HS

(Hussain and Thakur, 1963)

RESULTS

During 2016-17, three advanced lines viz. RBN-13017, RBN-13022 and RBN-13029 exhibited moderately resistant response with 22.33%, 18.42% and 13.76% disease incidence respectively under rating 2 whereas two advanced lines i.e. KN-294 (34.56%) and 14CBN009 (42.99%) expressed a moderately susceptible response (rating 3). Similarly, four varieties or advanced lines namely KN-279, 14CBN001, Faisal Canola and Rohi Sarson showed susceptible response with (66.54%), (54.28%), (64.77%) and (60.12) percent disease incidence (rating 4) while KJ-159 was used as spreader that expressed highly susceptible response with 92.14 % disease incidence under rating 5 (Table 2). During 2017-18 four varieties/ advanced lines namely KN-279, 14CBN001, Faisal Canola and Rohi Sarson exhibited susceptible response with (68.84%), (56.38%), (66.97%) and (58.22) percent of disease incidence (rating 4) whereas two of the advanced lines i.e. KN-294 (31.86%) and 14CBN009 (40.09%) showed moderately susceptible response (rating 3). Similarly, three advanced lines viz. RBN-13017, RBN-13022 and RBN-13029 exhibited moderately resistant response with 24.63%, 19.82% and 12.06% disease incidence respectively under rating 2 (Table 3).

Table 2. Response of Brassica varieties/ advanced lines to *A. brassicae* under natural field conditions during 2016-17

Sr. No.	Varieties/ Advanced lines	Disease Incidence	Rating	Response
1	RBN-13017	22.33h	2	MR
2	RBN-13022	18.42i	2	MR
3	RBN-13029	13.76j	2	MR
4	KN-279	66.54b	4	S
5	KN-294	34.56g	3	MS
6	14CBN001	54.28e	4	S
7	14CBN009	42.99f	3	MS
8	Faisal Canola	64.77c	4	S
9	Rohi Sarson	60.12d	4	S
10	KJ-159 (Spreader)	92.14a	5	HS

*The Mean values in a column sharing similar letters do not differ significantly as declared by the LSD test (P ≤ 0.05).
R = Resistant, MR = Moderately resistant, MS = Moderately susceptible,
S = Susceptible, HS = Highly susceptible.

Table 3: Response of Brassica varieties/ advanced lines to *A. brassicae* under natural field conditions during 2017-18

Sr. No.	Varieties/ Advanced lines	Disease Incidence	Rating	Response
1	RBN-13017	24.63h	2	MR
2	RBN-13022	19.82i	2	MR
3	RBN-13029	12.06j	2	MR
4	KN-279	68.84b	4	S
5	KN-294	31.86g	3	MS
6	14CBN001	56.38e	4	S
7	14CBN009	46.09f	3	MS
8	Faisal Canola	66.97c	4	S
9	Rohi Sarson	58.22d	4	S
10	KJ-159 (Spreader)	94.34a	5	HS

*The Mean values in a column sharing the similar letters do not differ significantly as determined by the LSD test (P ≤ 0.05). R = Resistant, MR = Moderately resistant, MS = Moderately susceptible,
S = Susceptible, HS = Highly susceptible.

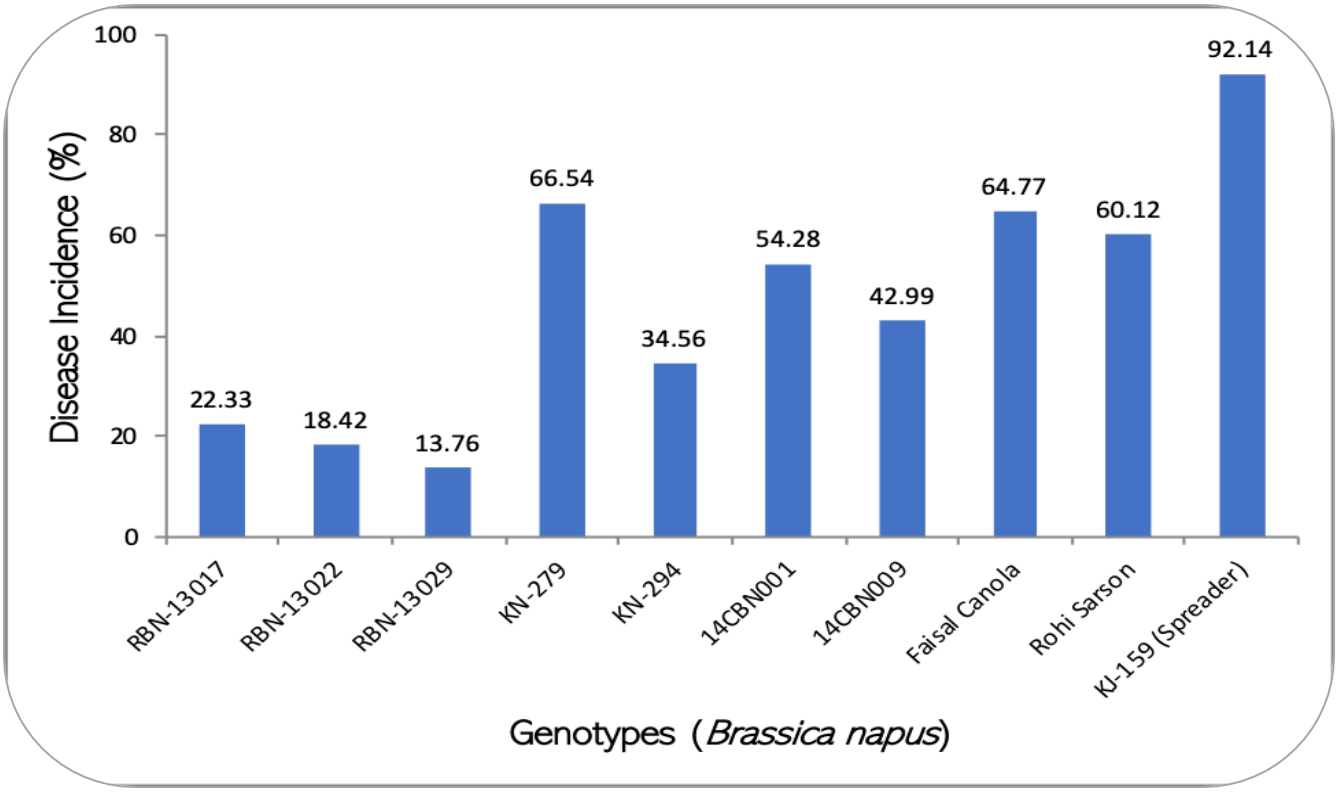

Figure 1. Alternaria leaf spot disease incidence (%) on various Brassica napus genotypes during year?

DISCUSSION

Alternaria leaf spot disease of Rapeseed mustard is caused due to numerous species but *A. brassicicola*, *A. brassicae* and *A. japonica* are more important species that play a pivotal role in reduction of oil quality as well as seed yield quantity (Verma and Saharan, 1994). *A. brassicae* caused huge yield losses of 47-50% in mustard under conducive and favourable conditions (Meena *et al.*, 2016). The pathogen decreases photosynthetic rate, shatters the premature pods, enhances senescence and shrivelled seeds production are the distinctive or distinguished symptoms of the disease (Shresta *et al*., 2000*).* The characteristic symptoms of *A. brassicae* at early stages appear on lower and older leaves, subsequently become visible on stem and pods of mustard plants. Likewise, tan colour small spots appear that consistently increase in size and unite to form a lesion. These numerous lesions on pods and leaves abruptly diminish the photosynthetic rate as a consequence (Kubota *et al.,* 2003*)*. Likewise, dark lesions can be visualized or seed on stem during seedling stage of rapeseed mustards whereas brown to black spots on hypocotyl also decreased normal plant growth (Valkonen and Koponen, 1990). *A. brassicae* can survive at 25-35ₒC whereas optimum temperature is 30ₒC for mycelial growth on host plant or under controlled conditions. Similarly, RH also play a significant role in mycelial development and sporulation (Meena *et al.,* 2008).

The resistant status of rapeseed plants based on mutation breeding techniques is costly (Harloff et al., 2012) whereas screening through convention breeding under field conditions is durable, climate resilient, long lasting in the fields and appropriate solution against the disease attack. Therefore, in the current research ten varieties/ advanced lines of rapeseed germplasm viz. RBN-13017, RBN-13022, RBN-13029, KN-279, KN-294, 14CBN001, 14CBN009, Faisal Canola, Rohi Sarson and KJ-159 (Spreader) were used for screening against Alternaria Leaf Spot disease. It was observed that three advanced lines viz. RBN-13017, RBN-13022 and RBN-13029 expressed moderately resistant response with 22.33%, 18.42% and 13.76% disease incidence respectively whereas two advanced lines i.e. KN-294 (34.56%) and 14CBN009 (42.99%) showed moderately susceptible response (rating 3). Similarly, during subsequent year three advanced lines viz. RBN-13017, RBN-13022 and RBN-13029 exhibited moderately resistant response with 24.63%, 19.82% and 12.06% disease incidence respectively while four varieties/ advanced lines namely KN-279, 14CBN001, Faisal Canola and Rohi Sarson

showed susceptible response with (68.84%), (56.38%), (66.97%) and (58.22) percent disease incidence. The findings of the current research are in line with the studies of Subhani *et. al.,* (2018) who used 15 cultivars of rapeseed i.e Toria Selection-A, Punjab canola, Faisal canola, Bulbul 98, Oscar, BSA, Rainbow, CON III, DGL, Legend, CON II, Dunkeld, Excel, Shirale, Cyclone and observed that Punjab canola expressed moderately susceptible response in field conditions. Likewise, Hussain *et al.* (2018) also used ten advanced lines/varieties viz. 8CBN001, 8CBN002, 10CBN003, 10CBN005, 11CBN001, 11CBN003, 11CBN009, 11CBN011, 12CBN003 and Chakwal Sarsoon under field conditions through split plot design and observed that advanced line 10CBN005 expressed shattering tolerance while 8CBN002 exhibited susceptibility as compared to others promising genotypes of rapeseed. Similarly, Bisht *et. al.,* (2015) screened 240 Brassica germplasm under field conditions against most important diseases of rapeseed mustard (Brassica spp.) and seven genotypes namely IC-326253, IC-255498, IC-339589, IC-296685, IC-417020, IC-335847 and IC-339597 showed resistant response against aforesaid diseases.

CONCLUSION

It is concluded that the moderately resistant varieties or advance lines of rapeseed mustard found by screening against *Alternaria brassicae* in current research trial might be useful in the future programs of breeding to develop a resistant commercial genotypes which could be released for commercial cultivation if they expressed the other desirable characteristics.

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Contribution of Authors:

Muhammad R. Bashir	:	Collected advanced lines of rapeseed germplasm, performed the experiment,
Abid Mahmood	:	Designed the study, supervised the experiments and helped in manuscript writing.
Muhammad Atiq	:	Designed the study, supervised the experiments and helped in manuscript writing.
Muhammad Yaseen	:	Analyzed the data
Nasir A. Rajput	:	Designed the study, supervised the experiments and helped in manuscript writing.
Muhammad Aftab	:	Help in conducting experiment.
Ahsan M. U. Din	:	Help in conducting experiment.
Muhammad Mohsan	:	Help in conducting experiment.
Qamar A. T. Khan	:	Help in conducting experiment.