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Components of slow mildewing in oat powdery mildew caused by *Blumeria graminis* f. sp. *avenae*

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Abstract

Powdery mildew of oat (*Blumeria graminis* f. sp. *avenae)* is the most deleterious foliar disease of cultivated oat, particularly in cooler regions. Slow mildewing, a partial resistance behaves genetically as a quantitative trait and can be assessed by comparing with mildew development on a highly susceptible cultivar grown under the same conditions. The slow mildewing components (disease severity, AUDPC and infection rate) under field conditions were studied in 15 moderately susceptible along with highly susceptible check (HJ-8) selected from panel of 303 field screened oat genotypes. The lines were categorized into slow mildewing categories based on terminal disease severity and AUDPC values. The AUDPC values of these lines ranged from 475 (JPO-45) to 1775 (HJ-8) and infection rate ranged from 0.053 to 0.144, being minimum in JPO-45 and maximum in HJ-8. All the tested lines gave 3 or 4 infection type under greenhouse evaluation and were designated as moderately susceptible and susceptible genotypes. Three oat lines showing moderately susceptible reactions, along with HJ-8 were selected to carry out experiment on slow mildewing in greenhouse. Among all the selected lines, the incubation period varied between 3-4 days and latent period between 4-5 days, i.e. maximum of 4 and 5 days, as compared to minimum of 3 and 4 days in HJ-8, respectively. After 11 days of inoculation, countless conidiophores bearing conidia were produced on all the lines except in IG-03-203. The size of powdery mildew colony was recorded and maximum colony size 3.87 mm was observed in HJ-8 followed by 2.87, 3.37 and 3.30 mm in IG-03-203, JPO-20 and KRR-AK-06, respectively.

Key words: Oat, powdery mildew, area under disease progress curve (AUDPC), infection rate, slow mildewing

Oat (*Avena sativa* L.) belonging to family Poaceae is a multipurpose cereal crop grown in *Rabi* season in many parts of the world. Oat is frequently grown as dual-purpose crop. In India it is mainly used for fodder, however whole grain consumption as a dietary source for human consumption is also increasing (Sangwan *et al.* 2012). Oat can easily be grown during winter period because of its good regeneration capacity, drought and cold tolerance ability. Among many diseases, Powdery mildew (*Blumeria graminis* D.C. (Speer) f. sp. *avenae* Em. Marchal) is the most deleterious foliar disease of cultivated oat, particularly in cooler regions. An estimated loss of up to 32 per cent in yield due to this pathogen has been reported (Jones *et al.* 1987). In Himachal Pradesh, powdery mildew was observed severe and severity up to 95 per cent was recorded in oat during 2015-16 and 2016-17 (Banyal *et al.,* 2016; Anonymous, 2016; Anonymous, 2017). Severe loss of green fodder yield (41.44%) and grain yield (35.06%) has been recorded due to powdery mildew (Anonymous, 2016). Since, oat is fodder as well as whole grain crop, it is not preferred to spray chemicals as crop is grown for green fodder purpose and chemicals will get into food chain directly affecting animal and human health. Using resistant varieties can reduce use of chemicals during the vegetation season. Race specific resistance is overcome by change in the virulence pattern of the pathogen and resistant varieties

become susceptible within short period of time whereas, race-nonspecific resistance is generally inherited quantitatively, often more durable and provides partial or slow mildewing resistance (Chen 2005; Sthapit *et al.* 2012). Slow mildewing, a partial resistance behaves genetically as a quantitative trait and can be assessed by comparing with mildew development on a highly susceptible cultivar grown under the same conditions. No studies have been conducted on components of slow mildewing in oat powdery mildew till date. Therefore, the present investigation constitutes the first study on slow mildewing components of oat powdery mildew caused by *B. graminis* f. sp. *avenae*.

Materials and Methods

Susceptible genotypes having slow disease progress as compared to highly susceptible cv. (HJ-8) were selected from field screened lines for studying components of slow mildewing in field conditions (Disease severity, AUDPC and infection rate) and greenhouse conditions (incubation period, latent period, number of conidiophores bearing conidia and size of mildew colonies).

Evaluation of genotypes for slow mildewing

Evaluation of genotypes under field conditions:

The 303 oat genotypes were sown in Research farm of Department Plant Pathology, CSK HPKV, Palampur in 1m line with 50 cm spacing in between the lines. Fifteen moderately susceptible lines belonging to different disease severity categories and one highly susceptible check HJ-8 were selected for studying slow mildewing components (AUDPC and infection rate) under natural epiphytotic conditions in the field. The per cent disease severity was recorded at fortnight intervals and area under disease progress curve (AUDPC) and infection rate (r/day) were calculated and correlated with the disease severity. The AUDPC was calculated using the formula given below by Shaner and Finney (1977):

$$AUDPC = \Sigma (y_i + y_{i+1}) / 2 \times (t_{i+1} - t_i)$$

where, y_i = Disease severity at time t_i

y_{i+1} = Disease severity at time t_{i+1}

Relative AUDPC was calculated by comparing actual AUDPC values with the value of susceptible check HJ-8 as per formula given below:

Relative AUDPC= (AUDPC value of the genotype ×100)/ AUDPC value of check

The infection rate (r) of the same genotypes was also calculated by using the equation given by Vander Plank (1963):

$$r = \frac{2.3}{t_2 - t_1} \times \text{Log}_{10} \frac{X_2 (1-X_1)}{X_1 (1-X_2)}$$

where, X_1 = Proportion of infected tissues at time t_1;

X_2 = Proportion of infected tissues at time t_2.

Relative infection rate was calculated by comparing actual infection rate with the value of susceptible check HJ-8 as per formula given below:

Relative infection rate= (infection rate of the genotype ×100)/ infection rate of check

The lines were categorized as slow mildewers on the basis of terminal disease severity under field conditions as per the categories given by Saari and Prescott (1975). The detail is as under:

Category size/ terminal disease severity (%)	Category no.
15.1-20	I
20.1-30	II
30.1-40	III
40.1-60	IV
>60	V

Evaluation under greenhouse conditions

Fifteen oat genotypes along with susceptible check HJ-8 were sown in iron trays and 7 days old seedlings were then transferred in the chambers having single colony purified culture of pathogen. These lines were dusted with the inoculum and the trays were surrounded with susceptible HJ-8 pots for creating inoculum load for proper infection. The observation on infection types under compound stereoscopic microscope was recorded after 10 days of incubation as per the descriptions given by Banyal *et al.* (2005), when leaves of susceptible cv. HJ-8 were fully covered with powdery mass (on 10th day after inoculation). The description of scale for recording infection types under *in vitro* evaluation is as below:-

Infection Type	Description
0	No mycelium growth
1	Sparse mycelium growth with very little sporulation
2	Slight growth of mycelium is evident macroscopically. Microscopically slight to moderate growth of mycelium with conidiophores of the fungus
3	Moderate growth of mycelium is evident macroscopically. Microscopically moderate development of mycelium with moderate to heavy sporulation is seen
4	Abundant growth of mycelium is evident macroscopically. Microscopically abundant development of mycelium with heavy to very heavy sporulation is visible

Study of slow mildew components

To study the components of slow mildewing, 7-day old seedlings were inoculated by spraying the inoculum of *B. graminis* f. sp. *avenae* which was prepared by suspending conidia in water containing traces (0.0025%) of Tween 20. Seedlings were inoculated by spraying conidial suspension which contained 20±2 conidia per microscopic field (40X) using an atomizer (Chaudhary and Banyal, 2016). After inoculation, the seedlings were incubated in an isolation chamber in greenhouse under 16 hr photoperiod / 8hr darkness. Each pot contained 10 to 15 seedlings and each treatment had four replications. Once the colonies of powdery mildew were visible, the leaves were cut from base on the 5th day and were floated on water in Petri dishes for further observations under stereozoom microscope. Observations on disease development were recorded in the selected lines on 5, 6, 8 10 and 11th day of inoculation on the components of slow mildewing.

To record data on colony size and number of conidiophores bearing conidia per colony, the individual powdery mildew colonies on the detached leaves of seedlings were marked with a coloured marker by observing the leaves under stereozoom microscope. On each leaf, three colonies were marked and each line had three inoculated leaves. Observations on various components of slow mildewing were recorded on these marked colonies as follows:

Incubation period: For studying incubation period, inoculated leaves of each genotype were observed daily for the appearance of first macroscopically visible colonies.

Latent period: For studying latent period, inoculated leaves of each genotype were observed daily for the initiation of conidial development from the very first day of inoculation.

Size of colonies: The size of a colony was ascertained by measuring its average diameter from two sides in mm (the longest line passing through the centre of the colony) by scale under stereoscopic microscope after every 24 hours upto 11 days after inoculation.

Number of conidiophores bearing conidia: The number of conidiophores bearing conidia per colony were counted using a stereoscopic microscope upto 11 days.

Results and Discussion

Evaluation of genotypes under field conditions

Slow mildewing under field conditions were studied on 16 susceptible oat genotypes selected from panel of 303 lines. These lines were selected on the basis of terminal disease severity and AUDPC values. The values of AUDPC ranged from 475 to 1775, being lowest in JPO-45 and highest in susceptible check HJ-8. Similarly, infection rate (r/day) ranged from 0.053 to 0.144/day, being lowest in JPO-45 and highest in HJ-8. The lines were categorized into slow mildewing categories based on terminal disease severity (Table 1). The line JPO-45 was categorized into Category II and 5

lines i.e. ADG-96, HFO-52, HFO-163, IG-03-208 and OATS-79 into Category III, 5 lines i.e. IG-03-203, JPO-20, KRR-AK-06, JO-25 and JO-35 into Category IV and 5 lines i.e. PO-1, Sohzar, JPO-822, OL-1861 and HJ-8 were categorized into Category V. The values of AUDPC again grouped theses lines in the same slow mildewing categories as observed on the basis of terminal disease severity. It was observed that AUDPC value of JPO-45 belonging to Cat-II was 475 whereas, the values of lines grouped in Cat-III and Cat-IV ranged from 605 to 655 and 735 to 1075, respectively, however it ranged from 1110 to1775 in Cat-V having highly susceptible lines. Correlation between slow mildew categories and infection rate did not differentiate the groups. Infection rate of line belonging to Cat-II was 0.053/day, where as it ranged from 0.068 – 0.085 and 0.066 – 0.087 for Cat-II and Cat-III, respectively. The infection rate of Cat-V was clearly different with the values ranging from 0.105 to 0.144. The infection rate of Cat-II and Cat-V were distinguishable; however, infection rate values of Cat-III and Cat-IV fell between same range and were unable to distinguish these groups. The terminal disease severity also ranged from 30 to 95 per cent, being highest in HJ-8 (95%) and lowest (30%) in JPO-45.

Green house evaluation of genotypes

The fifteen lines along with susceptible check HJ-8 which were selected in field screening were also screened for their reaction against single colony purified inoculum of *B. graminis* f. sp. *avenae* maintained in greenhouse. All the lines gave 3 or 4 infection type for the single colony purified inoculum and were designated as moderately susceptible and susceptible genotypes (Table 1). Out of 16 lines, 6 lines showed moderately susceptible reaction to pure culture of the pathogen and 3 lines *viz.* IG-03-203, JPO-20 and KRR-AK-06 from this set of 6 lines were selected randomly for further evaluation of slow mildewing components.

Slow mildewing components

Three oat lines, KRR-AK-06, IG-03-203 and JPO-20 showing moderately susceptible reaction to single colony purified powdery mildew inoculum along with susceptible check HJ-8 were selected to carry out experiment on study of slow mildewing components. Among all the lines, the incubation period varied between 3-4 days and the latent period between 4-5 days. Maximum incubation period of 4 days was observed in IG-03-203, JPO-20 and KRR-AK-06 and minimum 3 days in HJ-8. Similarly, higher latent period of 5 days was also observed in these lines as compared to 4 days in susceptible check (HJ-8). After 5 days of inoculation with *B. graminis* f. sp. *avenae* inoculum, minimum conidiophores bearing conidia i.e. 8 were observed in IG-03-203 followed by 16 and 14 in JPO-20 and KRR-AK-06 respectively, whereas conidiophores bearing conidia per colony was maximum (22) in HJ-8. After 6 days of inoculation, conidiophores bearing conidia ranged from 14-35 in selected lines with least *i.e.* 14 in IG-03-203 followed by 26 in JPO-20, 24 in KRR-AK-06 and being maximum of 35 in HJ-8. On 8th day after inoculation, number of conidiophores bearing conidia were found to be 30 (IG-03-203), 40 (JPO-20), 35 (KRR-AK-06) and 50 (HJ-8). After 10 days of incubation, except HJ-8 all the lines had countable number of conidia i.e. 49 (IG-03-203), 60 (JPO-20), 55 (KRR-AK-06) and HJ-8 had a greater number of conidia that were unable to count and was designated as countless (C). After 11 days, countless conidiophores bearing conidia were produced on all the selected lines except in IG-03-203 whereas, being very high numbers in susceptible check (HJ-8). The size of powdery mildew colony was recorded after 11 days of inoculation and the data were presented in Table 2. Maximum colony size *i.e.* 3.87 mm was observed in HJ-8 followed by 2.87, 3.37 and 3.30 mm in IG-03-203, JPO-20 and KRR-AK-06, respectively (Table 2). The values of AUDPC on these four lines ranged from 735 to 1775 and maximum value 1775 was found in HJ-8 followed by 1075 in JPO-20, 1050 in KRR-AK-06 and minimum 735 in IG-03-203. Infection rate (r/day) values in the selected 4 lines ranged between 0.071 to 0.144/day. The maximum infection rate (r/day) 0.144/day was observed in HJ-8 followed by 0.071/day in JPO-20, 0.087/day in KRR-AK-06 and 0.09/day in IG-03-203 (Table 1 & 2).

On the basis of slow mildewing categories, values of AUDPC under field conditions and observations on slow mildewing components under greenhouse conditions, genotypes IG-03-203, JPO-20 and KRR-AK-06 were found slow mildewers as compared to susceptible check HJ-8. The low values of AUDPC, high incubation and latent period, smaller size of colonies, less sporulation as represented by number of 'conidiophores bearing conidia' per colony in selected

Table 1. Field and green house evaluation of oat genotypes to study slow mildewing components

Sr. No	Variety	Terminal disease severity	Field evaluation					Greenhouse evaluation	
			AUDPC		Infection rate (r)		Slow mildewing category	Infection type	Reaction type
			AUDPC value	Relative AUDPC	r per day	Relative infection rate			
1.	ADG-96	40	605	34.08	0.085	58.71	Cat-III	3	MS
2.	HFO-52	40	640	36.06	0.068	47.10	Cat-III	4	S
3.	HFO-163	40	655	36.90	0.085	58.71	Cat-III	4	S
4.	IG-03-203	42	735	41.41	0.090	60.62	Cat-IV	3	MS
5.	IG-03-208	40	615	34.65	0.085	58.71	Cat-III	4	S
6.	JPO-20	60	1075	60.56	0.071	49.48	Cat-IV	3	MS
7.	JPO-45	30	475	26.76	0.053	36.88	Cat-II	3	MS
8.	KRR-AK-06	60	1050	59.15	0.087	60.18	Cat-IV	3	MS
9.	OATS-79	40	635	35.77	0.085	58.71	Cat-III	3	MS
10.	PO-1	72	1210	68.17	0.105	72.64	Cat-V	4	S
11.	JO-25	45	700	39.44	0.091	63.44	Cat-IV	4	S
12.	JO-35	45	755	42.54	0.066	46.16	Cat-IV	4	S
13.	SOHZAR	70	1175	66.20	0.126	87.67	Cat-V	4	S
14.	JHO-822	76	1125	60.85	0.085	58.71	Cat-V	4	S
15.	OL-1861	70	1110	62.54	0.120	83.21	Cat-V	4	S
16.	HJ-8	95	1775	100.00	0.144	100.00	Cat-V	4	S

Table 2. Slow mildewing components on selected oat genotypes

S.No.	Genotypes	Slow Mildewing Components											
		In vitro							Field evaluation				
		Incubation Period (Days)	Latent Period (Days)	Number of Conidiophores bearing Conidia					Colony Size (mm)	AUDPC	r (Infection rate/day)	Relative AUDPC	Relative infection rate	Slow mildewing category
				5th day	6th day	8th day	10th day	11th day						
1	IG-03-203	4	5	8	14	30	49	60	2.87	735	0.09	41.41	60.62	Cat -IV
2	JPO-20	4	5	16	26	40	60	C	3.37	1075	0.071	60.50	49.48	Cat -IV
3	KRR-AK-06	4	5	14	24	35	55	C	3.30	1050	0.087	59.15	60.18	Cat -IV
4	HJ-8	3	4	22	35	50	C^{+}	C^{++}	3.87	1775	0.144	100.00	100.00	Cat -V
	CD (P=0.05)	**-**	**-**	**-**	**-**	**-**	**-**		**0.20**	**158.05**	**0.017**			

- = No conidia

C = Countless: too many to be counted; more than (>) 75, but give individual appearance

C^{+} = Countless: conidiophores and conidial density very high.

C^{++} = Countless: conidiophores and conidial density very high and look like cluster overlapping each other

*Figures within parentheses are arc sine transformed values

cultivars as compared to highly susceptible check HJ-8 clearly supported the result that, the cultivars IG-03-203, JPO-20 and KRR-AK-06 were slow mildewers.

It is well known that incubation period, latent period, size of colonies, sporulation are important components of rate reducing resistance (Parlevliet 1979). Shaner and Finney (1977) evaluated effect of nitrogenous fertilizers on expression of slow mildewing in knox wheat genotypes. They considered AUDPC, apparent infection rate and time required for severity to reach 10 per cent and found that AUDPC had a lower error variance and was a superior measurement of slow-mildewing. Conner *et al*. (2003) while studying impact of powdery mildew on grain yield on soft white cultivars found that the response of cv. Fielder to powdery mildew has not dramatically changed since 1981 and suggested that this cultivar might have a race-nonspecific disease resistance. They concluded that the slow buildup of powdery mildew in cv. Fielder, AC Reed and AC Nanda was similar to the response of winter wheat cultivars with a slow mildewing resistance. Chaudhary and Banyal (2016) studied slow mildewing in selected pea lines based on observations on incubation period/latent period, size of colonies, sporulation as represented by numbers of 'conidiophores bearing conidia' per colony, AUDPC and infection rate. Harneet (2019) categorized 755 lines of wheat collected from NBPGR, CIMMYT and European entries into slow mildewing categories based on terminal disease severity. They categorized 71 lines into Category I, 293 into category II, 192 lines into Category III, 164 lines into Category IV and 25 lines into category V and suggested that these genotypes can be deployed in areas where race specific resistance is broken by the pathogen.

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