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COMPARATIVE VIRULENCE IN ISOLATES OF *TILLETIA INDICA* AND HOST RESISTANCE AGAINST KARNAL BUNT OF WHEAT

H. M. Zia Ullah, M. I. Haque, C. A. Rauf, L. H. Akhtar* and M. Munir

Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi
*Guar Botanist, Agricultural Research Station, Bahawalpur
Corresponding author email: ziaghazali@gmail.com

ABSTRACT

Wheat that becomes unacceptable for human consumption, emitting fishy smell of trimethylamine due to karnal bunt of wheat (*Tilletia indica*), affects the international wheat trade extensively, owing to quarantine restrictions on germplasm movement across the borders. Applying boot inoculation technique, 39 host wheat genotypes of varied pedigrees were, inoculated with teliospores collected from various isolates separately, obtained from seven districts of Southern Punjab. Differences of disease reactions among the all test genotypes as well as of virulences among the isolates were highly significant. There was also a significant genotype-isolate interaction indicating the gene-for-gene relationship among the host and the pathogen. Isolates collected from Lodhran (Kbm) and Khanewal districts (Kbj), were more virulent followed by isolates of Bahawal Nagar (Kbi), Bahawal pur (Kbs), Rahim Yar Khan (Kbw) and Multan (Kbp) districts respectively, while the virulence capacity of the isolate from Vehari region (Kbn), in their comparison was the lowest of all as characterized by susceptibility category. Significant positive correlation between percent infection and extent of fungal colonization existed. Genotypes/cultivars Punjnad-1, V032862, V033010, V056041, V056132, V066211, V066213, Shafaq-06 and Kiran were classed as resistant against the mixture of isolates, while Inqilab-91, Fareed-06, FSD-85, Bwp-79, Satluj-86, Uqab-2000, AS-2002, Manthar-03, Lasani-08, Chakwal-50, Sahar-06 and Bwp-2000 exhibited moderately susceptible and susceptible response. No cultivar was found immune to karnal bunt of wheat. The possible increase in the virulent patterns of diverse forms of the pathogen stresses to continue conventional breeding strategies for genetic resistance, while the variability analysis of isolates of *Tilletia indica* will support to explore new sources of resistance in bread wheat.

Key words: Karnal bunt, comparative virulence, *Tilletia indica*

INTRODUCTION

Wheat being an important crop of the zone sown in the months of November-December and harvested in April having short statured, early maturing attributes, spares the field for the next crop in the of April i.e. much earlier of the onset of rainy season which results in least favorable environmental settings for the pervasiveness of the plant pathogens. Irrigated plan lands of South Punjab (Pakistan) are the sub tropical warm areas of the country with high temperature and low humidity.

Yield and production constraints are associated with several biotic and abiotic stresses. Karnal bunt (*Tilletia indica)* first reported from the former state of Karnal, India (Mitra, 1931) and later on in many countries of world geographic canvas, has now become a threat in Pakistan. During a survey of 78 localities in the province of Khyber Pakhtunkhwa, the highest disease incidence up to 46.0% was observed on WL-711 grown in Hathion. Disease incidence on Pak-81 and Pirsabak-85 was recorded up to 6.32% and 5.57% respectively, while district wise incidence was observed as Mangora (2.53-23.42%), Mardan (0.41-46.00%), Malakand (6.32%), and 0.54-5.85% in Swabi (Ehsan-Ul-Haq *et al*., 2002). Regions in south of the Punjab marked as free zone from karnal bunt (Bhutta *et al*., 1999) has now been, reported with above 40% sample infection in the wheat samples collected from the grain markets of various cities and towns (Shakoor *et al*., 2008) of the province. This has gained grounds for changing the prior trends set for the teliospores epidemiology. Environmental fluctuations support the surveillance and prevalence of pathogen of karnal bunt of wheat. Dry and arid zone of the south Punjab differing not distinctly with their temperature regimes, have been proving equally a substrate for the *Tilletia indica* inoculum. No extensive studies at any department level, on this disease have yet been made in these wheat growing areas. However individual efforts by some workers reflects the alarming situations of threatening increase in the incidence level of karnal bunt of wheat in almost all cultivated varieties of bread wheat grown in these districts. The current studies were conducted on the basis of intensity level and extent of damage to look at the existence of differential pathogenic virulences among the various isolates of *Tilletia indica* collected from wheat growing areas of South Punjab and to determine host- pathogen interaction with the advanced cultivars of bread wheat.

MATERIALS AND METHODS

Samples of diseased wheat spikes were collected from seven districts. Fungal mass cultures prepared from affected grains of these samples were further categorized in to isolates viz isolate KBi; prepared from samples received from district Bahawal Nagar (BNG), Isolate KBj from district Khanewal (KWL), isolate KBm from district Lodhran (LDN), isolate Kbn from district Vehari (VHR), isolate Kbp from district Multan (MTN), isolate Kbs from Bahawalpur (BWP) and isolate Kbw developed from samples of district Rahim Yar Khan (RYK).

Wheat germplasm: Thirty nine germplasm accessions (Table 2) were taken from Economic Botany Section-Regional Agricultural Research Institute, Bahawalpur. These included commercial wheat varieties and advance lines of breeding program, with better genetic potential and characteristics for yield components, morphologically and economically. These lines had the good capacity to endure harsh weather conditions of the region.

Inoculation for artificial epidemics: Fresh sporidial suspension derived from Teliospores populations obtained from each one year old sample preliminary considered an isolate, collected from various vicinities was used, and inoculum was prepared according to the method of Bonde *et al.,* (1996). Pericarp of the infected grains was ruptured and teliospores of the pathogen *T. indica* were detached and scrapped off the bunted kernels, shaken well in solution of a detergent Tween-20 and water with a ratio of 3-4 drops of Tween-20 in 100 ml of water for 20 seconds. Solution was then centrifuged at 3000 rpm, sieved through 100 m sieve to remove the kernel debris from the suspension. Sodium hypochlorite (1 %) was added to the solution for surface sterilization and centrifuged at 3000 rpm for about 2 minutes. One to two (1-2) drops of the teliospore suspension was added to the petri dishes containing water agar medium These plates were incubated at 21 $\pm$ 2 C^0 for about 15 days for pure culture preparation of T. indica by streaking them on agar slants of another set of petri plates prepared with pure sterilized water agar. Colonies of the fungus were developed in 9-10 days which were cut into small pieces and were made stick to the lower side of the lid of the freshly prepared PDA in 100 ml Erlenmeyer flasks and also in some petri plates. Small amount of sterilized distilled water was added to the both culture medium. This process supplemented much to the discharge and liberation of the secondary sporidia which were later on incubated at 20°C for 20 days for mass multiplication of sporidial suspension.

1ml (10,000 sporidia /ml) of allantoid sporidial suspension was injected by syringe method, at boot stage, in to boot cavity of eight to ten spikes/entry of each accession for each isolate as this technique gives maximum percentage of infection (Chona *et al.,* 1961; Singh & Krishna, 1982; Aujla *et al.,* 1980). Maximum possible humidity was provided to the inoculated heads by irrigating the fields just after inoculation. Bunted heads were harvested at the time of maturity and were threshed manually.

Table 1. Standardization of vulnerability category based on CI value.

Co-efficient of Infection (CI)	Susceptibility Category
0	Highly resistant (HR)
0·1 – 5.0	Resistant (R)
5·1 – 10.0	Moderately Susceptible (MS)
10·1- 20.0	Susceptible (S)
20·1 and above	Highly Susceptible (HS)

(Aujla *et al*., 1989)

Data for disease incidence were collected by including the total number of infected seeds and healthy grains in the inoculated spikes in all treatments, to compare the percent incidence among the test genotypes as well as the comparative differential response against the diverse isolates. To evaluate the susceptibility category, all genotypes inoculated with the mixture of isolates were categorized (Table 1) on the basis of CI, following the susceptibility category given by Aujla *et al*. (1989).
Completely randomized design was used with three replications for all the treatments. The collected data were subjected to ANOVA applying methods of Gomez and Gomez (1984) on computer program MSTAT-C (Michigan State University, 1996). The treatment means were compared by standard error method computed as $s/\sqrt{n}$, where s stands for standard deviation while n denotes the number of observations.

RESULTS AND DISCUSSION

All isolates could be differentiated on the basis of their differential reactions on each accession. Virulences among the isolates differed significantly high (Table 3). Percent incidence caused by various isolates showed variety of susceptibility categories in the all cultivars/lines. Analysis of variance shows that significant variation exists among the genotypes with respect to their genetic potentials against the KB virulences. The isolates collected from the districts of Lodhran and Khanewal proved to be the most virulent among all, followed by isolate collected from district of Bahawalnagar. While pathogenically, the least virulence was observed in the isolate of Vehari district.

Table 2. Susceptibility category of each host lines including commercial cultivars, based on coefficient of infection when inoculated with mixture of isolates.

Genotypes	Grains infected	Total No. of Grains	% infection	0	1	2	3	4	Gross Total	C.I
INQ-91	77	503	15.31	426	45	25	5	2	29.5	5.86
Uqab-2000	157	479	32.78	322	112	26	14	5	56.5	11.8
PND-1	40	411	9.73	371	37	2	1	0	11	2.68
AS-2002	262	617	42.46	355	245	14	2	1	70.75	11.47
BK-2002	204	392	52.04	188	189	10	3	2	56.5	14.41
Manthar-03	282	707	39.89	425	215	45	20	2	93.25	13.19
Fareed-06	118	455	25.93	337	87	24	6	1	39.25	8.63
Lasani	146	303	48.18	157	98	33	11	4	53.25	17.57
Faisalabad-85	101	390	25.9	289	68	29	2	2	35	8.97
Chakwal-50	182	364	50	182	144	14	19	5	62.25	17.1
Blue Silver	196	360	54.44	164	123	41	16	16	79.25	22.01
V03 2862	28	254	11.02	226	23	4	1	0	8.5	3.35
V03 3010	23	294	7.82	271	9	6	4	4	12.25	4.17
V04 5006	50	374	13.37	324	22	4	21	3	26.25	7.02
V05 6007	104	467	22.27	363	56	34	12	2	42	8.99
V05 6037	308	539	57.14	231	278	24	4	2	86.5	16.05
V05 6038	182	524	34.73	342	171	10	1	0	48.5	9.26
V05 6041	43	428	10.05	385	36	4	3	0	13.25	3.1
V05 6132	40	417	9.59	377	27	9	4	0	14.25	3.42
V06 6205	192	615	31.22	423	176	14	2	0	52.5	8.54
V06 6211	66	403	16.38	337	60	3	2	1	19	4.71
V06 6213	76	417	18.23	341	73	2	1	0	20	4.8
V06 6237	204	483	42.24	279	177	25	2	0	58.25	12.06
V06 6238	205	569	36.03	364	162	36	7	0	63.75	11.2
V06 6240	325	614	52.93	289	265	45	15	0	100	16.29
V06 6253	225	412	54.61	187	201	21	2	1	63.25	15.35
V06 6284	282	708	39.83	426	270	9	3	0	74.25	10.49
V06 6301	146	403	36.23	257	119	23	4	0	44.25	10.98
V06 6302	311	666	46.7	355	241	56	12	2	99.25	14.9
WL-711	401	527	76.09	126	268	76	43	14	151.25	28.7
V06 6305	199	583	34.13	384	161	25	10	3	63.25	10.85
V06 6309	91	460	19.78	369	25	21	44	1	50.75	11.03
BWP-79	88	541	16.27	453	22	35	28	3	47	8.69
Sahar-06	110	479	22.96	369	14	56	33	7	63.25	13.2
Shafaq-06	56	610	9.18	554	24	12	14	6	28.5	4.67
Kiran	51	614	8.31	563	14	14	21	2	28.25	4.6
Satluj-86	67	413	16.22	346	26	24	13	4	32.25	7.81
V06 6303	237	546	43.41	309	174	36	15	12	84.75	15.52
BWP-2000	185	550	33.64	365	141	34	8	2	60.25	10.95

The significant genotype- isolate interaction indicates the presence of vertical resistance and there is gene for gene relationship in host-pathogen system (Van der Plank, 1968). Genotypes possessing the gene for resistance /susceptibility against the isolates verily responded with the varying intensities. Commercial varieties/cultivars included in the set showed the inheritance for resistance against the pathogen. Due to boot inoculation, least genetic potential was observed as the maximum success of infection in this regard is achieved. Studying the segregation pattern in the generations, Sharma *et al*. (2004) suggested that two independently segregating, dominant genes jointly confer the KB-free attribute.

Variety/lines PND-1, V032862, V033010, V056041, V056132, V066211, V066213, Shafaq-06 and Kiran were ranked as resistant, while Uqab-2000,AS-2002, BK-2002, Manthar-03, Lasani, Chakwal-50,V05 6037,V06 6237, V06 6238,V06 6240,V06 6253,V06 6284,V06 6301,V06 6302,V06 6305,V06 6309, Sahar-06, V06 6303, BWP-2000, Blue silver and WL-711 showed response as susceptible and highly susceptible (Table 2). Indian variety WL-711 unanimously recognized as susceptible check in karnal bunt screenings (Dhaliwal, 1995; Satvinder & Nanda, 2002), showed

Table 3. Analysis of variance showing significant differences among virulences of the isolates, as well as among the genetic host resistances against *Tilletia indica*. Significant GxI indicates the existence of host – pathogen interaction.

S.O.V.	*d.f.*	*S.S.*	*M.S.*	*F-Cal*
Genotype	38.00	52636.37	1385.17	11.93**
Isolates	6.00	26818.78	4469.80	38.51**
G x I	228.00	40296.54	176.74	1.52*
Error	546.00	63376.37	116.07	
	818.00			

* = Significant ** = Highly Significant

Table 4. Percent incidence of karnal bunt on different host line with standard error of mean, inoculated with various isolates of *Tilletia indica* separately indicating the pathogenic and resistance capacities of isolates and genotypes respectively.

Accessions	Kbi	Kbj	Kbm	Kbn	Kbp	Kbs	Kbw
INQ-91	18.71 ± 3.19	14.44 ± 0.48	15.77 ± 2.23	16.61± 3.98	13.06 ± 3.39	25.22±12.47	18.27 ±4.99
Uqab-2000	25.74 ± 7.42	28.55 ± 7.13	28.46 ± 9.13	15.23 ± 4.44	12.76 ± 3.31	31.75±11.35	28.35 ± 4.38
PND-1	3.59 ± 1.91	18.75 ± 3.52	7.29 ± 1.82	16.98 ± 3.71	3.68 ± 0.40	2.76±1.38	2.70 ±1.39
AS-2002	31.90 ± 6.59	34.76 ± 6.78	30.64± 9.19	19.00 ± 5.69	15.48 ±1.27	15.88±4.14	13.23 ±5.55
BK-2002	31.46 ± 10.46	45.71 ± 7.46	39.24±11.49	10.79 ± 2.59	20.64±12.36	8.26±3.26	16.23 ±2.03
Manthar-03	43.44 ± 1.55	38.63 ± 3.26	30.87± 1.07	6.64 ± 2.24	20.85±11.40	13.69±4.29	12.01 ±3.39
Shafaq-06	2.60 ± 1.51	6.72 ± 3.47	10.18± 4.07	7.21 ± 3.71	7.92± 3.21	6.29±4.24	7.56 ±3.83
Lasani	47.99 ± 1.75	44.69 ± 2.20	36.32± 1.81	12.87± 3.06	26.78±13.33	18.62±8.05	24.87 ±4.84
Faisalabad-85	25.51 ± 5.75	26.65 ± 1.03	25.23± 4.21	15.77± 5.27	34.04 ±1160	16.17±3.11	29.74 ±8.21
Chakwal-50	17.59 ± 9.28	43.65 ± 8.75	32.94±11.01	21.40 ± 3.95	19.48 ± 2.14	14.04±2.40	25.57±6.61
Blue Silver	38.29 ± 4.31	52.60 ± 3.55	47.02± 5.43	34.36 ± 8.10	26.24 ± 3.25	37.33±8.93	26.54 ±2.94
V03 2862	8.02± 6.32	6.01± 1.89	5.86±1.34	1.98 ± 0.44	5.22 ± 2.32	5.86±2.66	16.63±7.04
V03 3010	10.80 ± 1.19	9.43 ± 2.51	16.47 ± 8.68	8.11± 1.44	11.09 ± 3.61	17.84±9.28	20.85±12.30
V04 5006	7.99 ± 4.13	11.99 ± 0.69	33.20 ± 1.15	3.87± 1.54	22.00±15.63	20.04±11.39	16.20 ±6.23
V05 6007	22.44 ± 0.50	21.49 ± 0.51	24.99 ± 5.90	11.46 ± 3.35	25.69 ± 5.74	23.00±7.32	18.26 ±5.49
V05 6037	29.90 ± 12.32	43.25 ± 2.96	41.50 ± 5.24	16.37± 3.15	11.21 ± 2.15	42.77±12.33	19.24 ±5.57
V05 6038	30.24 ± 4.94	34.17 ± 0.46	27.41±11.02	16.10 ± 2.50	11.01 ± 1.45	34.68±11.52	21.70 ±2.90
V05 6041	11.49 ± 6.71	13.48 ± 4.10	7.69±3.85	9.96 ± 3.65	4.87 ± 4.21	6.35 ±1.85	4.61 ±2.65
V05 6132	12.33 ± 7.03	6.22 ± 2.37	27.58 ± 12.19	3.13 ± 0.92	23.39±16.89	16.85 ±9.79	11.82 ±4.88
V06 6205	32.26 ± 10.63	37.14 ± 3.92	38.14 ± 4.14	9.25 ± 1.34	18.40 ± 8.17	30.13±10.56	15.73 ±4.86
V06 6211	26.27 ± 7.79	31.51 ± 2.82	42.16 ± 6.24	15.93 ± 2.72	12.83 ± 3.72	42.95±17.49	22.76 ±1.81
V06 6213	21.94 ± 8.54	26.25±8.83	38.93 ± 5.72	16.93 ± 2.71	11.49 ±1.91	23.78 ± 5.29	30.94 ±9.75
V06 6237	36.99 ± 5.23	45.24±1.54	33.61 ± 6.45	12.97± 2.0	17.02 ± 8.11	11.11± 4.79	14.74 ±3.73
V06 6238	39.64 ± 8.06	42.90±6.72	45.03±10.59	16.55 ± 2.84	20.35 ± 8.07	14.54 ±1.15	22.03 ±4.06
V06 6240	40.73 ± 2.84	49.24±2.19	32.21±11.08	14.36 ± 3.78	14.93 ± 3.59	20.90 ±1.30	18.35 ±3.04
V06 6253	31.67 ± 3.12	43.38±8.42	40.62±12.19	15.40 ± 3.46	12.56 ± 2.09	17.28 ±2.63	15.75 ±1.89
V06 6284	29.64 ± 5.85	32.28±4.34	29.12 ± 8.23	9.75 ± 1.95	10.48 ±1.16	15.01 ±3.27	11.74 ±1.29
V06 6301	25.01 ± 8.07	29.77±6.06	33.21±6.72	9.22 ± 1.62	13.45 ± 4.27	13.64 ±1.87	19.17±5.20
V06 6302	36.26 ± 1.72	43.19 ± 2.38	28.85 ± 8.89	15.82 ± 3.96	16.69 ± 4.63	38.84±10.63	18.82 ±1.77
WL-711	54.30 ± 8.57	63.68 ± 6.50	51.74±7.63	27.71 ± 6.70	53.09±10.85	42.16 ±7.98	46.25 ±0.73
V06 6305	28.48 ± 4.22	30.20 ± 3.73	29.32 ±7.74	17.05 ± 3.32	13.03 ± 2.43	18.35 ±1.16	17.47±4.55
V06 6309	21.99 ± 2.06	22.43 ±3.77	34.13±10.36	14.43 ± 3.06	25.30 ± 4.67	20.96 ± 2.99	18.01±6.14
BWP-79	11.32 ± 0.62	14.42 ±1.14	27.63 ± 6.78	12.10 ± 3.83	19.66 ± 7.97	15.97 ± 3.20	19.20 ±4.91
Sahar-06	13.32 ± 2.84	19.99 ± 2.68	32.36 ± 9.11	11.75 ± 2.28	13.42 ± 2.15	15.53 ±1.61	25.36 ±6.97
Fareed-06	17.03 ± 4.26	13.86 ± 0.82	21.32±10.87	16.67± 3.31	16.59 ±1.00	22.89 ± 3.17	22.98 ±1.73
Kiran	2.17 ± 0.62	22.74 ± 4.33	16.93 ±2.68	0.89 ± 0.38	4.23 ± 3.53	6.34 ± 4.23	16.99 ±2.69
Satluj-86	17.01 ± 2.05	16.10±0.23	13.00 ± 0.68	15.80 ± 3.42	15.99 ± 3.20	18.80 ±2.72	21.30 ±5.14
V06 6303	39.13 ± 6.79	37.36±5.00	25.68±8.77	13.47± 3.10	16.40±3.21	23.34±11.18	20.87±1.31
BWP-2000	32.42 ± 5.75	37.52±3.04	29.41±5.76	13.26 ± 2.71	19.90±1.38	21.18±11.26	19.92±0.85

highly susceptible reaction in our studies. Distinct disease incidence was pragmatic on different host lines with the inoculation of different isolates. Kbi, and Kbp caused less than 5% incidence on Punjnad-1, V066211 and kiran; whereas Blue silver, BK-2002, Manthar-03, Lasani, Faisalabad-85, V056007, V066238, V066309 and WL-711 gave reaction of more than 20% incidence (highly susceptible), inoculated with the similar isolates, indicating that both posses the different genes for resistance against karnal bunt infection (Table 4). Still the same lines V032862 and V056132 on the other hand, were susceptible in their reaction (23.73% and 27.58% respectively) against KB infection when inoculated with isolate Kbm. Low incidence of disease on Kiran and V066211 with inoculation of isolate Kbn and Kbw, was observed as 0.89% and 0.92% respectively, while on Blue Silver, high incidence of 34.36% and 26.54% was observed with the same isolates. Isolate Kbj caused the highest incidence on Blue silver (52.60%) and WL-711(63.68%); while Kbp and Kbn caused lowest infection 26.24% and 27.71% on the same most susceptible cultivars, respectively. These results match with the findings of Sharma *et al*. (2001) & Sharma *et al*. (2004) who categorized ten isolates of *Tilletia indica* into various pathotypes based on their differential reaction, collected from various locations in plains of north India and Zone I of Himachal Pradesh, India, on a set of 20 genotypes of wheat and triticale showing variable degree to resistance of Karnal bunt. They also found that some isolates were more virulent and showed high aggressiveness in causing the incidence, as compared to the others. Varieties/lines ranked with similar category of their susceptibility, in our study, advocate that, either there is no pathogenic differences exist among those particular isolates or there is no resistance gene in those genotypes that could have responded to them. Similar findings have earlier suggested by Bonde *et al*. (1996), while studying the four isolates obtained from separate field collections. No cultivar /line showed immune response to any of the isolates. They reported that this rare category came with the ratio of eight out of 20,000 entries when a continuous screening studies were carried out for 10 years; still including very low infection and not the immune all eight. In our studies, all commercial cultivars vastly sown in these regions including INQ-91, Uqab-2000, BK-2002, Manthar-03, Lasani, Faisalabad-85, AS-2002, Sahar-06, and Fareed-06 etc were susceptible in their reactions against the pressure of inoculum specially with boot inoculation methodology that allows to yield the maximum ratio of successful infection (Beniwal *et al.,* 2001). However, under natural field conditions, these cultivars behave as resistant varieties due to high temperature and low rain fall in the south. In addition to this, varieties also show their morphological (field) resistance (Royer & Rytter, 1985; Warham, 1988, 1990). (Riccioni *et al.,* 2006) when inoculated with spray inoculation techniques or exposed to the air borne infection of the disease because of the relative differences between morphological and physiological susceptibility (Riccioni *et al*., 2008). Presence of less resistance gene against KB virulences, in almost all commercial cultivars, is well compensated with the unfavourable environmental conditions in the area. With the low populations of the pathogen, the probability of booming infection also becomes low even under favorable environmental conditions because of what is known as the Allee effect (Smilanick *et al.,* 1989). More over, a little period in the wheat physiological system permits the infection to occur and, during that critical period of two to three weeks, the environmental conditions have to be favorable for the disease development. Threats for the epidemics could not be ignored as the pathogen exists and disease prevails at the hot spots.

A positive correlation between the % incidence and co-efficient of infection was observed. Percent incidence being an independent variable, after calculating the intensity of seed colonization, determines the susceptibility category under which a test genotype falls. Jafari *et al*. (2000) also reported significant positive correlation between coefficient of infection and percentage of infected grain for each entry. While working with nine isolates of *Tilletia indica,* Pannu and Chahal (2000) also reported a positive correlation between secondary sporidial production and disease incidence. Using the regression analysis, a trend line was extended in a chart beyond the actual data to predict future values. Value of R^2 shows more than 85% dependence upon incidence of disease while other factor contributes up to 15% that uses a simple linear trend line clearly indicating a rising trend (Fig. 1).

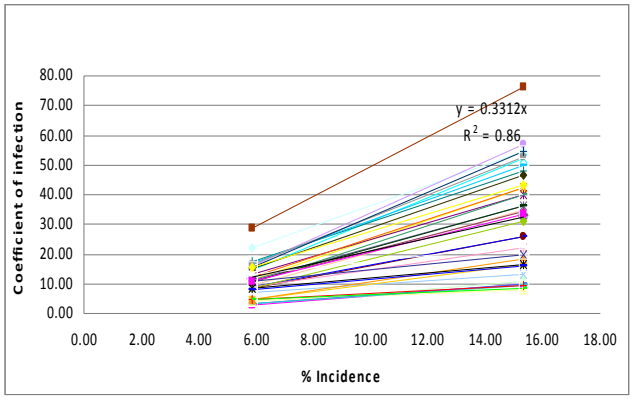

Fig.1. Series in rows showing the trend line indicating the relationship between coefficient of infection and % incidence. Regression equation expresses the multiple of x value (% incidence as an independent variable) to yield the seed colonization for calculation of susceptibility category.

Difference among the isolates prevails with respect to the frequency of virulence/avirulence alleles at different pathogenicity loci, corresponding to the resistance genes in the host lines (Datta *et al*., 1999). However, the study derived from the diagnostics by identifying the molecular markers based on pathogenicity loci will prove more confirmatory to the isolate characterization.

Apprehending the critical significance of breeding, the present study do emphasizes the need of continuous hybridization programs for genetic resistance, among the bread wheat so as to combat rapidly the changing and emerging trends in the pathogenic behaviuors of the pathogens.

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