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Pathogenic variation among isolates of *Tilletia indica* the causal organism of karnal bunt of wheat

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Key words: *Tilletia indica*, Karnal bunt, Pathogenic variation, Resistance, Wheat.

Abstract

Pathogenic variability among the twenty one isolates of *Tilletia indica* collected from 11 districts of Punjab and 4 from KPK. The response of some recent commercial varieties was also studied. Our study confirms the existence of pathotypes or aggressive types in isolates of *T. indica* in nature. As PB-25 was significantly more aggressive than all other isolates and showed moderately aggressive response with maximum mean coefficient of infection (6.03) while PB-03 was least aggressive. Based on pathogenic behavior on different varieties, isolates can be divided into two major groups. Among 12 commercial wheat varieties NIFA-Barsat, Punjab-2011, BARAS-09 and Seher-06 showed resistant to highly resistant response to all the isolates.

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Introduction

Wheat (*Triticum aestivum* L.) is a basic staple food for many societies (Curtis *et al.,* 2002). The area under cultivation in Pakistan is 8805 thousand hectares with annual production of 24.2 million tons and average yield is 2750Kgs/ hectares (Govt. of Pakistan, 2011). Diseases contribute as one of the major aspects to the yield losses of wheat (Jones & Clifford, 1978). Karnal bunt is one of the major fungal diseases of wheat in Pakistan. Though Karnal bunt never cause severe yield losses but it occurrence is reported always causing concerns intermittently. It is alternatively known as partial bunt of wheat caused by *Tilletia indica* (Mitra, 1931). Karnal bunt *(Tilletia indicia)* reduces the weight of grains, depreciates its quality and makes it revolting for human consumption (Gopal & Sekhon, 1988). As it is one of the quarantine diseases causing economic losses and affect international trading (Datta *et al*., 2000).

Germination, tillering and yield of wheat are extremely reduced by Karnal bunt (Jatav *et al.,* 2003) due to complete loss of embryo during the infection. Teliospores germinate only when environmental conditions are favorable for disease development (Rattan and Aujla, 1992; Dhiman *et al*., 1984). Teliospores germinate to give rise a whorl of filiform primary sporidia (Rattan and Aujala, 1990) which then produce two types (filiform and allantoid) of secondary sporidia (Goates, 1988). Allantoid sporida are banana shaped and are infectious in nature while filiform sporida are the reproductive entity (Dhaliwal and Singh, 1988). Secondary allantoid sporidia are assumed to cause infection; when it gets fused with each other. Therefore, there are chances of occurrence of different populations which may differ in morphology, physiology and aggressiveness. The isolates which produced more number of secondary sporidia exhibited more virulent response .There is existence of pathotypes or aggressive types in isolates of *T. indica* in nature and population of *T. indica* have been divided in to various groups on the basis of aggressiveness (Thirumalaisaisamy, 2006; Pannu and Chahal, 2000; Aujla *et al*., 1989).

Control of Karnal bunt has now become a major concern all over the world including Pakistan. The pathogen of Karnal bunt is soil, seed and air-borne, it can penetrate locally into host plant, so application of fungicidal spray is very critical (Workneh *et al*., 2008). Cultural practices that reduce the Karnal bunt incidence, such as delayed sowing, reduced nitrogen fertilization and reduced planting density can only reduce Karnal bunt incidence to some extent (Rivera-Castaneda *et al*., 2001). Thus, the most successful, efficient and economic method of disease control is the use of varieties with genetic resistance (Aujla *et al*., 1982). The major issue in the development of resistance varieties is limited research done on the pathogenic variability of *Tilletia indica* existed in wheat varieties.

Therefore, the study was conducted to determine the aggressiveness analysis/pathogenic variability of different isolates of Karnal bunt in field and response of some new wheat varieties against *Tilletia indica* isolates.

Materials and methods

Collection, Isolation and multiplication of T. indica

Two hundred and twenty six samples were collected randomly from storage areas and markets of Punjab (Bahawalnagar, Bahawalpur, Chakwal, Faisalabad, Gujranwala, Jhelum, Lodhran, Multan, Rahim Yar Khan, Rawalpindi/Islamabad and Sahiwal) and Khyber Pakhtunkhwa (Charsada, Mardan, Peshawar and Sawabi) during a survey (Table 1).

Table 1. Isolates of *Tilletia indica* collected from different districts of Punjab and Khyber Pakhtunkhwa.

Sr. No.	Province	District	Host (variety)	Isolate
1	Punjab	Chakwal	BARS-2009	PB-1
2	Punjab	Jhelum	Sehr-2006	PB-2
3	Punjab	Taxila	Pak-81	PB-3
4	Punjab	Rawalpindi	Bakhtawar	PB-4
5	Punjab	Taxila	Shehzor-2007	PB-5
6	Punjab	Rawalpindi	Seher-2006	PB-7
7	Punjab	Jhelum	Pak- 81	PB-8
8	Punjab	Sahiwal	FD-4	PB-12
9	Punjab	Sahiwal	Watan	PB-13
10	Punjab	Sahiwal	INQ-91	PB-14
11	Punjab	Bahawalnagar	Aas-2011	PB-16
12	Punjab	Bahawalnagar	Sehr-2006	PB-17
13	Punjab	Bahawalnagar	Unknown	PB-18

Sr. No.	Province	District	Host (variety)	Isolate
14	Punjab	Faisalabad	3099	PB-23
15	Punjab	Faisalabad	Unknown	PB-25
16	Punjab	Bahawalpur	Faisalabad-08	PB-29
17	Punjab	Bahawalpur	INQ-91	PB-30
18	Khyber Pakhtunkhwa	Peshawar	Unknown	KP-1
19	Khyber Pakhtunkhwa	Charsada	Unknown	KP-2
20	Khyber Pakhtunkhwa	Mardan	Unknown	KP-5
21	Khyber Pakhtunkhwa	Mardan	Unknown	KP-6

A single infected seed was taken from each collected sample and was powerfully vortexed for two minutes in 10 ml of sterilized distilled water in 15 ml eppendrof tube to release teliospores. This spore suspension was passed through a 60µm sieve and then centrifuged at 12000 rpm for two minutes. After centrifugation the pellet was re- suspended in 1 % sodium hypochlorite solution for 30 seconds for surface sterilization.

The spores were washed three times with sterilized distilled water to remove sodium hypochlorite. The spores were first centrifuged and then the pellet was re-suspended in sterilized distilled water. Washed spores were plated on water agar media and incubated at 200C for 10 days. *T. indica* colonies appeared after 10 days of incubation and were examined under microspore for confirmation. Colonies form single teliospore of *T. indica* were cultured on PDA medium and from this purified culture for mass multiplication was made on PDA for inoculum production.

Aggressiveness analysis/Pathogenicity of T. indica isolates and response of wheat varieties

Each isolate was separately cultured, multiplied and maintained on PDA plates. For aggressiveness analysis and screening of wheat varieties the sporidial suspension of each isolate was prepared in sterilized distilled water. The inoculum concentration was adjusted to 4-5 × 10^4 spores ml^{-1}. Twelve commercial wheat varieties (from Punjab and Khyber Pakhtunkhwa) along with susceptible (WL-711) and resistance (HD-29) check (Table 2) were used.

Wheat varieties were planted at CDRP, NARC fields. Plants were inoculated at booting stage by boot inoculation method. Three spikes per variety were inoculated. One ml of freshly prepared sporidial suspension of *T. indica* was injected with a hypodermic syringe into the boot when the awns were just emerging. After inoculation spikes were properly tagged and covered with glycine bags to provide and maintain sufficient humidity for fungus to multiply within the ears (Aujla *et al.*, 1989).

Table 2. Commercial wheat varieties sowed in the field.

Sr. No.	Varieties
1	WL-711
2	Bakhtawar-92
3	Fakhre-Sarhad
4	NARC-2011
5	HD-29
6	Janbaz
7	Aas-2011
8	Tatara
9	AARI-2011
10	BARS-09
11	NIFA-Barsat
12	Dharabi-2011
13	Seher-06
14	Punjab-2011

The inoculated spikes were harvested and collected in paper bags at the time of maturity. The samples were threshed manually. The data was recorded in terms of incidence and coefficient of infection (CI) (Bonde *et al.*, 1996; Warham *et al.*, 1986; Aujla *et al.*, 1989). Value of coefficient of infection was calculated for the level of resistance and susceptibility of the germplasm. Then based on the CI value the varieties were categorized as follows. (Table 3)

Results and discussion

Aggressiveness analysis of T. indica isolates

Twenty one isolates tested on 14 different varieties exhibited different disease responses (Table 3). The isolate PB-25 was the most aggressive isolate, exhibited susceptible reaction on WL-711, HD-29, Janbaz, Bakhtawar- 92, Fakhre- Sarhad, Tatara, AARI- 2011 and Dharabi-2011. Isolate PB-2, KP-2 and KP- 6 gave susceptible reaction on WL-711 but did not exhibit infection on other varieties. Isolate PB-14 caused moderately susceptible reaction on HD-29 and Fakhre-sarhad while isolate PB-18 caused moderately susceptible reaction on Janbaz and susceptible on NARC-2011.

Isolates PB-12, PB-13 and KP-5 produce susceptible reaction on AARI-2011, NARC-2011 and Aas-2011 respectively.

Table 3. Categories of response on the basis of Coefficient of infection values.

Coefficient of infection	Susceptibility category	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)

Isolates PB- (1, 3, 4, 5, 7, 8, 13, 16, 17, 23, 29, and 30) and KP-1 did not cause infection on all varieties. On the basis of coefficient of infection values the isolates can be divided into two groups (Fig. 1). Isolate PB-25 (from Faisalabad) showed significantly high value of coefficient of infection (6.03), showing moderately aggressive response and comes under group I. The rest of the isolates showed weekly aggressive response and form group II with three subgroups (Table 4 & 5; Fig. 1). Group 2 A contains isolates PB-1, PB-2, PB-3, PB-4, PB-5, PB-7, PB-8, and PB-12. Group 2 B contains PB-13, PB-14, PB-16, PB-17, PB-23 and PB-18 whereas Group 2 C contains PB-29, PB-30, KP-1, KP-2, KP-5, KP-6, PB-25.

Table 4. Disease response of different wheat varieties to twenty one isolates of *Tilletia indica*.

Var.	PB 1	PB 2	PB 3	PB 4	PB 5	PB 7	PB 8	PB 12	PB 13	PB 14	PB 16	PB 17	PB 18	PB 23	PB 25	PB 29	PB 30	KP 1	KP 2	KP 5	KP 6
WL-711	HR	S	HR	HR	HR	R	R	HR	R	HR	R	R	R	HR	S	R	R	HR	MS	HR	MS
HD-29	R	R	R	R	R	R	HR	HR	HR	MS	R	R	HR	R	MS	HR	R	HR	HR	HR	HR
Janbaz	HR	HR	HR	HR	HR	HR	R	HR	HR	HR	R	R	MS	HR	MS	R	HR	HR	HR	HR	R
Bakhtawar-92	R	R	HR	HR	HR	R	R	HR	R	HR	HR	HR	R	R	HS	R	HR	HR	HR	HR	HR
Fakhre-sarhad	R	HR	HR	R	HR	R	R	R	R	S	R	R	R	R	MS	HR	R	R	HR	R	R
Tatara	HR	HR	HR	R	HR	HR	HR	HR	R	R	HR	HR	R	R	S	HR	HR	HR	HR	R	HR
NIFA-Barsat	HR	HR	HR	HR	HR	R	R	HR	HR	HR	HR	R	HR	R	HR	HR	HR	HR	HR	R	HR
AARI-2011	HR	HR	HR	HR	HR	HR	HR	MS	HR	HR	HR	R	HR	HR	MS	R	HR	HR	HR	HR	HR
Punjab-2011	HR	HR	HR	HR	HR	HR	HR	HR	R	HR	R	HR	HR	R	HR	HR	R	HR	HR	R	R
Aas-2011	R	HR	HR	HR	HR	R	HR	HR	R	R	R	R	HR	HR	R	R	HR	R	HR	MS	HR
NARC-2011	R	R	HR	R	HR	HR	R	HR	MS	R	R	HR	S	R	R	R	HR	HR	HR	R	R
BARS-09	R	HR	HR	HR	HR	R	HR	HR	HR	HR	HR	R	R	HR	HR	HR	HR	HR	HR	R	R
Seher	HR	HR	HR	HR	R	HR	HR	HR	HR	R	R	R	R	HR	R	HR	HR	HR	HR	HR	HR
Dharabi-2011	HR	HR	HR	R	HR	HR	HR	HR	HR	HR	R	R	HR	R	MS	HR	R	HR	HR	HR	HR

Similarly the isolates were categorized in to most aggressive (20.1 and above), aggressive (10.1-20.0), moderately aggressive (5.1-10.0), slightly aggressive (0.1-5.0) and non-aggressive (0) based on coefficient of infection.

Response of wheat varieties

The variety WL-711 (susceptible check) showed maximum coefficient of infection value (2.45) and significantly more susceptible response. Among the twelve varieties tested NIFA-Barasat, Punjab-2011, BARS-09 and Seher had low coefficient of infection values and showed resistant to highly resistant response to all the isolates. The rest of the varieties gave both susceptible and resistant response to various isolates. Janbaz, AARI-2011, Aas-2011, Dharabi-2011, these varieties showed moderately susceptible to highly resistant response. Fakhre-sarhad showed susceptible to resistance whereas Tatara showed susceptible to highly resistance response and Bakhtawar-92 showed highly susceptible to resistance response (Table 6).

Table 5. Response of twenty one isolates on fourteen wheat varieties.

Isolates	Coefficient of Infection (CI)*		Coefficient of Infection (CI)	Response on the basis of (CI)
PB-25	15.90^{a}	21.04^{a}	6.03a	Moderately aggressive
PB-18	12.21^{b}	14.10^{bc}	2.21^{b}	Weekly aggressive
PB-14	11.49^{bc}	12.84^{bcd}	1.49^{bc}	Weekly aggressive
PB-13	11.35^{bcd}	12.55^{bcde}	1.35^{bcd}	Weekly aggressive
PB-02	11.22^{bcde}	14.98^{b}	1.22^{bcde}	Weekly aggressive
PB-17	11.12^{bcde}	12.39^{bcde}	1.12^{bcde}	Weekly aggressive
PB-07	10.88^{cde}	12.30^{bcde}	0.88^{cde}	Weekly aggressive
KP-05	10.87^{cde}	11.81^{cde}	0.87^{cde}	Weekly aggressive
PB-23	10.76^{cde}	11.70^{cde}	0.76^{cde}	Weekly aggressive
KP-06	10.72^{cde}	11.68^{de}	0.72^{cde}	Weekly aggressive
PB-16	10.68^{cde}	12.08^{cde}	0.68^{cde}	Weekly aggressive
PB-01	10.60^{cde}	11.22^{de}	0.60^{cde}	Weekly aggressive
PB-29	10.57^{cde}	10.91^{de}	0.57^{cde}	Weekly aggressive
PB-30	10.53^{cde}	11.09^{de}	0.53^{cde}	Weekly aggressive
PB-05	10.52^{cde}	11.09^{de}	0.52^{cde}	Weekly aggressive
PB-12	10.49^{cde}	10.55^{de}	0.49^{cde}	Weekly aggressive
KP-02	10.36^{cde}	10.74^{de}	0.36^{cde}	Weekly aggressive
PB-08	10.35^{cde}	10.93^{de}	0.35^{cde}	Weekly aggressive
PB-04	10.32^{cde}	10.61^{de}	0.32^{cde}	Weekly aggressive
KP-01	10.14^{de}	10.17^{e}	0.14^{de}	Weekly aggressive
PB-03	10.08^{e}	10.16^{e}	0.08^{e}	Weekly aggressive
LSD (0.05)	1.25		1.25	

*Transformed data by adding 10.

Table 6. Response of wheat varieties based on coefficient of infection.

Varieties	Coefficient of Infection (CI)*	Coefficient of Infection (CI)
WL-711	12.45a	2.45a
Bakhtawar-92	11.72^{ab}	1.72^{ab}
Fakhre-Sarhad	11.67^{ab}	1.67^{ab}
NARC-2011	11.63^{ab}	1.63^{ab}
HD-29	11.55^{abc}	1.55^{abc}
Janbaz	11.05^{bcd}	1.05^{bcd}
Aas-2011	10.75^{bcd}	0.75^{bcd}
Tatara	10.71^{bcd}	0.71^{bcd}
AARA-2011	10.56^{cd}	0.56^{cd}
BARS-09	10.45^{d}	0.45^{d}
NIFA-Barsat	10.42^{d}	0.42^{d}
Dharabi-2011	10.42^{d}	0.42^{d}
Seher	10.42^{d}	0.42^{d}
Punjab-2011	10.32^{d}	0.32^{d}
LSD (0.05)	1.0213	

*Transformed data by adding 10.

Karnal bunt is one of the major quarantine concern diseases of wheat, which strictly affects the import and export of wheat grains. A number of scientists have conducted *T. indica* variability studies on the basis of morphological and physiological characteristics (Thirumalaisaisamy, 2006; Pannu and Chahal, 2000; Aujla *et al.*, 1989). The current study confirms the existence of pathotypes or aggressive types in isolates of *T. indica* in nature. Coefficient of Infection was calculated to check the pathogenic response of twenty one Isolates on fourteen wheat varieties. The most aggressive isolate (PB-25) was from Faisalabad whereas the least aggressive isolate (PB-3) was from Taxila (Table 4 and 5). The pathogen during annual cycle undergoes sexual stage and there is assortment of virulent genes and due to this the popultions of *T. indica* showed continuous variability. Although in our study isolate PB-25 was most aggressive but it showed low teliospore germination percentage and only filiform secondary sporidia, which are not known to cause high infection (Ingold, 1997; Mitra, 1935; Aujla *et al.*, 1987; Singh *et al.*, 1998; Kumar *et al.*, 2004). PB-25 can be used in disease screening programs/breeding to incorporate resistance. Based on pathogenic behavior on different varieties, isolates can be divided into two major groups. Isolate PB-25 (from Faisalabad) showed moderately aggressive response and falls under group I. The rest of the isolates showed weekly aggressive response and fall in group II with three subgroups (Fig. 1).

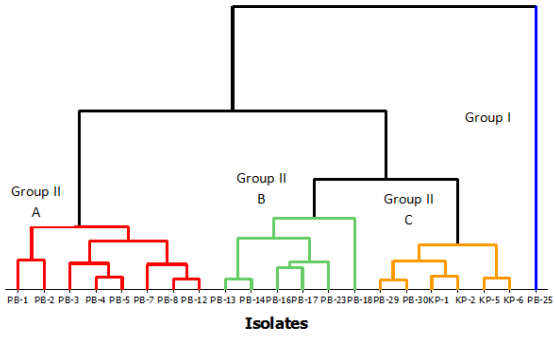

Fig. 1. Grouping of isolates based on coefficient of infection on fourteen varieties.

The variety WL-711 (susceptible check) showed maximum coefficient of infection value (2.45) and significantly more susceptible response.

Among 12 commercial wheat varieties NIFA-Barsat, Punjab-2011, BARS-09 and Seher showed resistant to highly resistant response to all the isolates (Table 6). These varieties need to be tested again in both field and glasshouse conditions to establish their effectiveness against Karnal bunt as disease escape is very common phenomena in this disease. Karnal bunt has a close relationship with the environmental conditions specific to that area and climatic conditions play an important role in disease establishment and spread (Stefanski *et al*., 1994). Similar results have been reported by (Warham, 1988; Kaur and Nanda, 2002).Since the results of present study showed the evidence of variability in the pathogen and so as the behavior of the different genotypes also showed varied response to the isolates. There is need to explore more of the areas in the wheat fields of the country in different ecologies. This will depicts the true scenario of variability of the pathogen and their response to some of the leading cultivars to be recommended for cultivation.

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