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Screening of Wheat (*Triticum aestivum L*) Genotypes against Karnal Bunt (*Tilletia indica*) under Arid Conditions

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IN WHEAT (*Triticum aestivum* L.), the production of disease resistant genotypes is crucial for managing diseases.Although, breeding programmes are challenging in disease-free areas owing to quarantine regulations but it is a necessity for upgrading the wheat genotypes that are adapted to current climate change situations. Karnal bunt caused by *Tilletia indica* has a significant influence on production and quality parameters of what as it adds a fishy odour and flavor to the wheat. A screening study was undertaken for Karnal bunt observation in two growing seasons in the high yielding area of South Punjab, i.e**,** Bahawalpur**.** Twenty one wheat commercial varieties developed in Regional Agricultural Research Institute Bahawalpur, Pakistan were screened for disease comparison between two wheat growing seasons (2019-2020 and 2020-2021). The area between the top of the spike and the surrounding boot sheath was inoculated with sporidial inoculum using a 3CC syringe with a 22-gauge needle. To assess the resistance/susceptibility of each line/cultivar of karnal bunt , a modified rating system was used. Disease incidence varied in cultivars during 2020-2021 growing season. In 2019-2020, Kohi Noor, Aas-09, Millat-11, and WL-711 had lower disease incidence than infected cultivars. While Shafaq-06, Johar-16, and Galaxy-13 have no disease incidence in the 2020-2021 . Overall, the disease occurance, persistence and severity is relatively low in Bahawalpur, that indicates arid environment is a favorable indicator for developing karnal bunt resistant genotypes. However, it is important that regular disease screening of Karnal bunt through monitoring at larger scale should be promoted in other wheat-growing areas as well for ensuring the adaptation/selection of disease resistant wheat genotypes.

Keywords: Commercial variety, Karnal bunt, Varietal variation, Wheat genotypes.

Introduction

Wheat is grown throughout the world with an annual production more than 600 million tonnes. Production of wheat must continue to increase annually by 2% to meet future demands imposed by population and prosperity growth. This challenge will be further compounded by increased temperature due to global warming (Fischer et al. 2002; Tesfaye, 2021). Wheat is among the most important crop and staple food in Pakistan (Ali et al., 2017) In Pakistan average wheat production 26 mounds per acre which is very low as compared to other vital wheat growing countries such as Egypt, France, India, and USA (Zulfiqar, 2010). There exists various factors that influence reduction in wheat yield such as pathogens, improper time of sowing, unhealthy seed and weed competition. The most important factor in this regard is the attack of pathogens. Karnal bunt caused by *Neovossia indica* is now emerging threat for all wheat growing regions of the world (Bishnoi et al., 2020).

Karnal bunt also known as partial bunt was first reported by Mitra et al. (1931) from Karnal,

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a place now in Haryana state of India from where its name originates. . Being a seed, soil and air borne pathogen, it mainly affects common wheat, durum wheat, triticale, and other related species making it the limiting factor in wheat export as the pathogen is regulated by most countries as a quarantine pest (Kumar et al., 2021). As a seed borne pathogen it threatens wheat trade between countries. Therefore, it has significance in seed certification aspects (Kumar et al., 2008; Tan et al., 2009).

Its spread and evolution depends on its lifecycle as the pathogen is heterothallic and undergoes sexual reproduction after teliospore germination (Turgay et al., 2020). Primary and secondary sporidia or hyphae as compatible mating types must fuse to form a dikaryon which enhances the chances of variation due to heterozygosity that plays a significant role in the production of new variants (Jones 2009). In both dry and semi-arid environments, humidity plays an important influence in pathogenicity (Kumar et al., 2021). Wheat is sensitive to the pathogen in semi-arid locations when high humidity levels (Sandhu et al., 2022). caused by rainfall coincide with heading stage, but in desert regions irrigation plays a major role in pathogen epidemiology (Iquebal et al., 2021; Kumar et al., 2021). The fungus, which is a floret-infecting disease, attacks ovaries in developing wheat heads, converting the seed into a black powdery mass of teliospores (Yadav et al., 2020), and significantly reducing the grain's quality and commercial viability.

The major impact of Karnal bunt of wheat is on quality and not on reduction. Infected seeds are the most important carrier of pathogens for trans-regional and long-distance dispersal of the spores (Bala et al., 2017; Gurjar et al., 2021). Contaminants of more than 1% have a negative impact on wheat quality and consumer acceptability, resulting in an unpleasant fishy odour and black discolouration, lowering quality and palatability. More than 3% contaminated seeds in a wheat lot renders it unsuitable for human consumption (Ullah et al., 2012), resulting in financial losses for the exporting nations (Kumari et al., 2020). The most effective management method for Karnal bunt would be the use of resilient wheat genotypes, which would also minimise the risk of the disease spreading to Karnal bunt-free areas. In India, Iran, Iraq, Mexico, Nepal, Pakistan, South Africa, and the United States, the illness has spread (Jones, 2009). The detection of the infection at an early stage is crucial for its investigation and control. When the pathogen is found in a country or region, quarantined restrictions are imposed, preventing international trade of seeds from such areas (Carris et al., 2006; Figueroa et al., 2018).

Most wheat-importing nations need a supplementary statement from exporting countries stating that the wheat shipment in question was grown in a KB-free environment (Gurjar et al., 2021). As a result, KB is a problem for the grain sector since it acts as a worldwide non-tariff restriction to wheat trading. The KB causal pathogen has a zero-tolerance limit throughout most wheat-importing nations.

Owing to the above mentioned disease problems and dire need to improve wheat germplasm through breeding, the current study was carried out in order to assess the occurance of of karnal bunt in commercial wheat varieties by comparing two-year Bahawalpur status and to discover resistant germplasm.

Materials and Methods

Screening of twenty-one cultivars was done against kernel bunt during two consecutive years 2019-20 and 2020-21 at the Regional Agricultural Research Institute, Bahawalpur. While, *in vitro* studies were performed at the Department of Plant Pathology. In field, the seeds were sown in the first week of November through drill sowing. The row to row distance was kept 30cm apart while the length of each plot was 2m. The experiment was laid out in RCBD design with three replications. The experimental area comprised of three blocks having seven varieties in each. During the research duration agronomic applications were kept same. At maturity, spikes were collected, threshed and evaluated for bunted grains. The pedigree of the varieties is shown in Table 1.

At maturity, the karnal bunt seeds were visually observed by selecting the germ side of kernel that showed dark color and gives fishy smell.

The coefficient of infection was determined by using the Table 2 given below.

TABLE 1. Pedigree of the varieties

Varieties	Pedigree
Aas 11	PRL/PASTOR//2236(V6550/SUTLEH-86)
Millat-2011	CHENAB2000/INQ-91
Punjab 2011	AMSEL/ATTILA//INQ-91/PEW'S'
Lasani 2008	LUAN/KOH-97
Seher 2006	CHILL/2* STAR/4/BOW//BUC/PVN/3/2*VEE#10
Shafaq 2006	LU 26/HD 21790/ 2*INQALAB 91
Fareed 2006	PT'S'/3/TOB/LFN//BB/4/BB/HD-832-5//ON/5/G-V/ALD'S'// HPO
Ujala	CGSS02B00125T-099B-099Y-099M-099Y-4WGY-0B
Galaxy-13	PB96/87094/MH97
WL=711	S 308 / Chris /Kalyansona

TABLE 2. Coefficient of infection to assess the susceptibility of wheat cultivars

Sr no.	Coefficient of infection	Susceptibility	Abbreviation
1	0	Highly Resistant	1R
2	0.1-5.0	Resistant	2R
3	5.0-10.0	Moderately Susceptible	1S
4	10.0-20.0	Susceptible	2S
5	20.0 to above	Highly Susceptible	3S

Survey report

During the experimental duration, a random survey of wheat fields in diverse areas of South punjab, Pakistan was carried out when the crop was on maturity stage. 5 spikes from each field were selected and placed in polythene bags and tagged properly with area name and collection date. The areas in Table 3 were visited, samples were collected as well as disease prevalence in the respective areas was calculated. The collected samples were brought to Plant Pathology department of regional agricultural research institute for further analysis. The survey was carried in order to know the prevalence of karnal bunt as well as collection of pathogen for extracting primary sporidia.

Preparation of teliospore suspension

Seeds were selected which had cracks and cleavage inside it and a black powdery sori appeared which are discharged by distilled water as torrent flow. The infected seeds were grounded and appropriate water was added and after that filtration was done in order to remove seed debris. The teliospores were obtained through centrifugation at 3000rmp during which teliospore settled down while supernant was removed; further, commercial bleach (5%) was used to disinfect the spores.

Infected kernels were placed in sterile distilled water to isolate teliospores. Teliospore suspension was then passed through a mesh sieve to filter the seed debris into plastic centrifuge tubes with caps. A few drops of 0.5% Sodium Hypochlorite were added to 5ml filtrate to prevent the spores from clinging to centrifuge tubes (Bonde et al., 1999). The filtrate contains teliospores, grain particles and other small particles. The suspension was then stirred in a centrifuge at 3000rpm and 4°C for 5min to form a pallet of teliospores. But this pallet has some quantity of grain particles inside it so suspend it in 0.5% sodium hypochlorite and then rinsed twice in distilled water.

TABLE 3. Detail of surveyed areas and disease prevalence

Sr no.	Area	District	Disease prevalence (%)
1	Fortabbas	Bahawalnagar	12
2	Khichi Wala	Bahawalnagar	11
3	Marot	Bahawalnagar	9.8
4	Head Saifan	Vehari	14.6
5	Zahir Peer	Rahim yar khan	8.7
6	Rahim Yar Khan	Rahim Yar Khan	9
7	Uch Shareef	Bahawalpur	10
8	Lodhran	Lodhran	11.3
9	Kahror Paka	Lodhran	11
10	Dunya pur	Lodhran	10.9

The spore suspension was then added to petri dishes that conyained plain agar medium. The water agar was made from 20g of agr dissolved in 1L of water. Further, the inoculated petri dishes were placed in controlled conditions of 14h day light-10h darkness at 15-20ºC for 25-30 days after which the teliospores germinated and primary sporidia was observed on surface of plain agar.

Then flood the fungal growth on agar media with distilled water and dislodged the primary and secondary mycelium with needle and filtrate was passed through a 60µm nylon mesh sieve to remove germinated sporidia and other residues. The filtrate was calibrated with a hemacytometer at X400 to get 4.1×10^4 to 2.8×10^5 total spores per millimeter.

Artificial inoculation of Teliospores

Suspension collected during the previous year was inoculated in 2019 having *Tellitia indica* was then inoculated into the plants at the booting stage. Sporidial inoculum was inoculum by 3CC syringe having 22 gauge needle into the space between the top of the spike and enclosing boot sheath. About 1ml of inoculum was injected into the secondary tiller and a 2CC syringe was injected into the boot of the primary tiller. While spikes already emerge were showered by inoculum by hand sprayer until liquid runoff through it.

The same process was repeated during the second year and infected kernels were used to isolate and prepare teliospore suspension for next year's inoculation. On a 0-5 modified rating scale, the severity of the disease was assessed. A modified rating system for karnal bunt was used to measure the amount of resistance/susceptibility of each line/cultivar, based on Aujla et al. (1989) and Bonde et al. (1996).

Percent disease incidence= (No.of bunted seed)/(total no.of seeds)×100

Life cycle of Tellitia indica

Teliospores germinate at the soil surface and produce haploid primary sporidia which is disseminated by wind or splashed to the surface of leaves. They germinate to produce mycelium and produce secondary mycelium which is blown to upper leaves and wheat heads and produces glumes through stomata. Mycelium grows down to glume and upper developing kernel. At, maturity teliospores are produced in large quantity and perpetuate to soil surface having 2×10^3 to 50.5×10^3 spores per cm^3 of soil.

Environmental impact on disease development

Environmental factors like temperature, humidity, rainfall, wind speed and precipitation play an important role in the development of disease, spore germination, spore dissemination and infection. Data was taken from the meteorological station from the Agronomy department of the institute (RARI) that shows high humidity and average temperature elevate the disease incidence in the two-growing season (Table 4). Moreover, A customized rating scale was employed to determine the intensity of the Karnal wheat bunt (Table 5).

Statistical analysis

The results were statically analyzed by using the statistical pakage'statistix version 8.0' in order to compare the means of results by Least significant difference at 0.5% probability level (Steel et al., 1997). As the genotypes were tested in three replications, A randomized complete block design was used and data was subjected to analysis of variance technique.

TABLE 4.The detailed meterological data

Month	Temperature (°C)		Humidity (%)	Rainfall (mm)
	Minimum	Maximum		
July, 2019	25	45	88	Traces
August, 2019	25	40	89	96mm
September, 2019	26	38	86	16mm
October, 2019	24	37	84	86mm
November, 2019	19	31	79	22mm
December, 2019	05	15	73	23mm
January, 2020	04	14	82	19mm
February, 2020	08	20	75	Traces
March, 2020	09	23	82	103mm
April, 2020	17	33	78	13mm
May, 2020	23	44	76	14mm
June, 2020	27	48	75	0

Month	Temperature (°C)		Rainfall (mm)	Humidity %
	Minimum	Maximum		
July, 2020	22	42	83	76
August, 2020	25	42	127	79
September, 2020	37	25	-	78
October, 2020	26	35	-	81
November, 2020	18	32	-	85
December, 2020	06	14	-	73
January, 2021	05	17	-	88
February, 2021	12	30	-	80
March, 2021	19	36	18	79
April, 2021	22	40	02	71
May, 2021	24	43	04	85
June, 2021	25	48	37	82

TABLE 5. A customized rating scale was employed to determine the intensity of the Karnal wheat bunt

Infection type	Symptoms	Coefficient of infection
0	Healthy kernals	0
1	Point infection developed 25% bunted seed	0.25
2	Point infection well developed 25% bunted seed	0.25
3	Infection along the groove 50% bunted seed	0.50
4	¾ seed converted to sorus 75% bunted seed	0.75
5	Complete conversion of seed into sorus 100% bunted seed	1.0

Results

The results of this study states that the locally developed wheat varieties which are now commercialized showed resistance against karnal bunt disease. During the experimental duration, in both years, i.e. In the years 2019-2020 and 2020-2021, similar genotypes were sown. The results demonstrate that all varieties were highly resistant to karnal bunt disease,however, nine were in category of resistant ones during 2020-2021 while seven varieties evaluated as resistant in 2019-2020. None of the varieties were identified as susceptible to the disease.

Lasani 8, Fareed-06, Punjab11, Johar-16, WL-711, Galaxy-13, Millat-11, Ujala-15, Kohi Noor, Sehar-06, Shafaq-06 and AAS-09 were the varieties that have bunted disease in both growing season (Table 6). In 2019-2021 Lasani 8 have high coefficient of infection of 0.5 while other varieties Fareed-06, Punjab 11, Ujala-15, Galaxy-13, and the varieties that shows low resistant varieties in that year was Sehar-06, Shafaq-06 and Johar-16 are resistant having coefficient of infection 0.3, 0.1, 0.175, 0.025 respectively.

In the growing season of 2020-2021 the diseased varieties having bunted seeds was Lasani 8, Fareed-06, Sehar-06, AAS-09, Millat-11, Kohi Noor, Punjab11, Ujala-15 and WL-711 (Table 6). There is varietal variation in disease incidence in the wheat season of 2020-2021. Kohi Noor, AAS-09, Millat-11 and WL-711 has different disease incidence than the diseased varieties in 2019-2020 (Fig. 1). While, Shafaq-06, Johar-16 and Galaxy-13 have no disease incidence in the growing season of 2020-2021. The total coefficient of infection in both growing seasons of wheat is 29.29 (Table 7).

TABLE 6. Reaction of wheat varieties against Karnal bunt disease during 2019-2020 and 2020-2021

Sr. No.	Varieties/ lines	Total grains	Disease reaction	Disease incidence (2019-2020)	Disease incidence (2020-2021)	Varietal disease incidence average percentage(%)
1	Fareed-06	1000	R	0.35	0.225	0-0.46 (46%)
2	Shafaq-06	1000	R	0.025	0	0-0.0125 (1.25%)
3	Sahar-06	1000	R	0.075	0.5	0-0.325 (3.25%)
4	Kohi Noor	1000	R	0	0.075	0-0.0375 (3.75%)
5	Mairaj-08	1000	R	0	0	0
6	Lasani-08	1000	R	0.5	0.275	0-0.6375 (63.75%)
7	F.S.D-08	1000	R	0	0	0
8	AAS-09	1000	R	0	0.025	0-0.0125 (1.25%)
9	Millat-11	1000	R	0	0.125	0-0.0625 (6.25%)
10	Punjab-11	1000	R	0.1	0.025	0-0.1125 (11.25%)
11	AARI	1000	R	0	0	0
12	NARC-11	1000	R	0	0	0
13	Gold-16	1000	R	0	0	0
14	Johar-16	1000	R	0.025	0	0-0.1125 (11.25%)
15	Galaxy-13	1000	R	0.175	0	0-0.0875 (8.75%)
16	Ujala-15	1000	R	0.1	0.075	0-0.1375 (3.85%)
17	R.D-1	1000	R	0	0	0
18	Pir Sabok-04	1000	R	0	0	0
19	WL-711	1000	R	0	0.025	0-0.1125 (11.25%)
20	Pir Sabok-05	1000	R	0	0	0
21	A.S-2002	1000	R	0	0	0

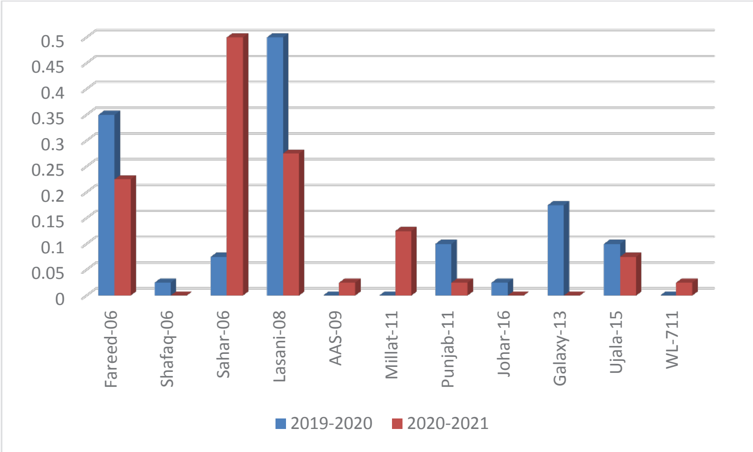

Fig. 1. Varietal coefficient of disease showing variation in both wheat growing season

TABLE 7. Calculating coefficient of infection of disease severity against karnal bunt

Scale	No. of value	No. of grains	Multiplication with num	Gross total	Total grains	Coefficient of infection
0	0	0	0	0	0	
1	0.25	26	6.5	6.5	26	
2	0.25	29	7.25	13.75	55	
3	0.50	9	4.5	18.5	64	
4	0.75	0	0	0	0	
5	1.0	0	0	0	0	
				18.75	64	C.I=29.29

Lasani-8 and fareed-06 have high disease incidence average percentage of 64% and 46% respectively. The low lying average percentage of disease incidence observed on Ujala-15, Kohi Noor, AAS-09 and Sehar-06. Furthermore, increasing temperature may decrease teliospore survivability in the soil, but increased wetness may lengthen the wheat development phase as well as yearly recurring infections caused by teliospores, resulting in larger reduction in yield. High moisture, on the other side, may enhance fatal germination, in which teliospores develop in the absence of a host, lowering inoculum concentration. Some picture from the study area and bunted wheat seeds are depicted in Fig. 2 (a,b).

Fig. 2 (a). Location map of the study site

Fig . 2 (b). Seeds affected from karnal bunt from experimental area

Discussion

Karnal Bunt existed since decades but it was not discovered outside of Asia until 1972, when it was discovered in Sonora, Mexico. The disease was isolated to Sonora's Yaqui and Mayo valleys at the period, and it was only found in small numbers in farmers' fields. Kumari et al. (2020) found that flour from grains with more than 3% bunted kernels had an odd colour and a foul odour. As a result, a modified rating system (Aujla et al., 1989) was utilised, with lines or varieties with 2.1-5 percent grain infection being deemed moderately susceptible, and cultivars with more than 10 percent infected grains being regarded very sensitive. It's not rare to find sources of resistance to Karnal bunt disease in wheat cultivars. The environment for Karnal bunt occurance is dry semi arid with moderate cold winters and occasional rainfalls. The similar conditions prevailed in our experimental site, however ability to withstand these conditions makes the actual resistance. However, during 2020-2021 wheat season rainfall was less as compared to 2019-2020 season. This may influence the disease incidence level. Bishnoi et al. (2020) stated that high precipitation, drought spells, increased CO_2 will elevate disease occurance.

Our results are similar to previous studies carried out by Khan et al. (2010) and Ullah et al. (2012) who conducted research in wheat and identified some resistant (PBW 502) and moderately resistant (Pastour, N-75-3 and N75-5) against karnal bunt. Some wheat and related genera (Aegilops) have been discovered to be resistant to bunt, and this resistance may be controlled for use in bread wheat (Khan et al., 2010; Ullah et al., 2012).

In the same district, Galaxy-13, Mixture, Fareed-06, and Sehar-06 had the highest disease incidence of 1.16%, 1.38 %, 3.24%, and 1.45%, respectively. Previously, the Karnal bunt was reported from Punjab (Bahawalpur, Bahawal nagar, Chakwal, Dera Ghazi Khan, Rawalpindi, Gujrat, Gujranwala, Jhang, Khanewal, Kasur, Layyah, Lodhran, Multan, Rawalpindi, Sahiwal, Sialkot, and Vehari) and Khyber Pakhtunkhwa (Mirza, 2005; Shakoor, 2009).

During the wheat season of 2012, the prevalence of kernel bunt on the types BARS2009, Seher-06, Pak-81, Bakhtawar-90, Faislabad08, Waten-94, Inqilab-91, and Aas-11 was surveyed in Chakwal, Rawalpindi, Jhelum, Sahiwal, Bahawal Nagar, Bahawalpur, and Faisalabad (Aasma et al., 2012).

The best technique to find disease-free regions for wheat production is to conduct frequent surveys in agricultural fields. Where they were planted, the continuous survey, certain wheat genotypes like Seher-06, and Galaxy-13 were susceptible . These have already been discovered to be highly sensitive to rust and spot blotch. The disease incidence is quite low in all the districts, which is a positive indication, and all the lines are resistant (Sajjad et al., 2018).

Because the diseases are present in wheat-growing regions in southern Punjab, it is critical to monitor the disease on a continuous basis and to perform a thorough study to identify karnal bunt disease-free areas in order to minimise future threats and to ensure the production of trade wheat.

Sthapit et al. (2014) stated the similar purpose of experiments, as landraces collection is vital for selection, identification and development of novel and genetically diverse disease resistant germplasm (Sthapit et al., 2014).

The reason for the disease dispersal and the neccessaity for its surveillance and assessment of genotypes is a pre requisite as its airborne as well as seed borne dispersal is critical (Aggarwal et al., 1999), As karnal bunt hotspot can shift from one season to another, at different places hence a more collarborative effort for disease management and varietal development makes such types of experiments an important aspects. Previous studies suggest that virulence can alter the genes during sexual reproduction which can further results in breakdown of races (Aggarwal et al., 2010; Singh et al., 1992).

Conclusion

The overall perception has healthy outcome, since despite the truth that diseases epidemiology levels are high, the varietal response does not reach the vulnerable level. The statistics clearly show that the climatic circumstances, as well as genetic sensitivity among the types planted in these areas, do not allow the disease to develop to its full potential. The possibility of an elevation in the pathogen's Resistant patterns underscores the need to continue traditional breeding tactics for genetic resistance, while the diversity analysis of *Tilletia indica* samples will contribute in the search for novel level of resistance in wheat varieties.

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