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The re-emerging Karnal bunt disease of wheat and preparedness of the global wheat sector

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Karnal bunt caused by smut fungus *Tilletia indica* (syn. *Neovossia indica* (Mitra) Mundkur), is one of the major fungal diseases of wheat that is considered to have a high potential of re-emergence, particularly in the North Western Plains Zone (NWPZ) of India[1]. It is named after the Karnal district of Haryana, where it was first discovered in 1931 by Manoranjan Mitra[2]. Although in the NWPZ Karnal bunt has been historically present in non-epidemic proportions, precipitation or high humidity at the flowering stage (February–March) may increase the infection percentage as high as up to 40%. During the post-harvest surveys conducted under the All India Coordinated Research Project on Wheat and Barley from the grain markets in 2019, 32.02% samples from a total of 7321 were found infected by *T. indica*. Maximum infected samples were reported from Haryana (56.69%), followed by Jammu and Punjab with 54.85% and 45.18% infected samples respectively. Fortunately, none of the samples collected from Madhya Pradesh, Gujarat, Maharashtra and Karnataka was found to carry the Karnal bunt disease[3]. Table 1 presents details of post-harvest sample survey for Karnal bunt for ten states in India, including the range of grain infection for each state. The percentage of infected samples is high; this should be a matter of concern for the Indian wheat sector and calls for stricter internal quarantine.

The rise in infection percentage in recent years has been primarily attributed to the adaptability of the pathogen to the prevailing weather fluctuations and absence of immunity in wheat mega-varieties. The extensive use of urea (nitrogen fertilizers) for higher yield and more irrigation events are also responsible for an increase in the intensity and incidence of Karnal bunt disease outbreaks. Karnal bunt is also reported to have a high propensity of becoming endemic in new geographies across Europe and Australia, which are at present free from this disease[4,5]. The associated risk becomes exceedingly important as *T. indica* is a fungus of high quarantine importance across the world with more than 70 countries having quarantine regulations imposed against it[6]. Therefore, the economic losses caused by the fungus should be understood in the form of a non-tariff barrier to the global wheat trade, rather than the direct yield losses which are minor only. The loss of yield due to Karnal bunt has been quantified to range between 0.01% and 1% in India and Mexico[4,7,8]. Owing to this, in the beginning, the disease was assumed to be of intermediate economic significance only[9]. The indirect losses caused by Karnal bunt also pertain to the rejection of wheat lots containing more than 3% of infected grains for human use[10]. Therefore, it is a peculiar wheat fungal disease that, unlike others (rusts, mildews, etc.), causes monetary losses to the growers more by affecting the quality and less by reduction in the quantity of the produce. Nonetheless, the studies of Murray and Brennan[11] and Stansbury and McKirdy[12] have shown that we should not be misled by the quantum of direct yield losses, as the former reported that an economic loss to the tune of AUD 490,900,000 per annum (17% of wheat economy) will accrue to the wheat commerce if *T. indica* gets an entry into Australia. Likewise, the latter study expected an even higher economic loss that could increase up to a significant 25%. The losses estimated included those due to reduction in yield, quality and the expected quarantine regulations that will be in place in case of such a scenario. The expected percentage of losses are enough

Table 1. Incidence of Karnal bunt disease in wheat in the 2018–19 crop season in India

State	Total samples	Infected samples	Infected samples (%)	Range of grain infection (%)
Punjab	2,809	1,269	45.18	0.1–12.14
Haryana	1,318	747	56.69	0.05–14.0
Rajasthan	300	123	41.0	0.1–21.9
Uttarakhand	1,189	58	4.88	0.1–5.0
Jammu	206	113	54.85	0.1–8.24
Uttar Pradesh	129	34	26.36	0.1–10.0
Madhya Pradesh	285	0	0	0
Maharashtra	341	0	0	0
Gujarat	692	0	0	0
Karnataka	52	0	0	0
Total	7,321	2,344	32.02	0.05–21.9

Source: ICAR-IIWBR[3].

for the wheat breeding programmes to consider Karnal bunt as a major bottleneck in wheat production and trade worldwide.

As global climate change has become a well-established fact, it should be expected to have a bearing on the evolutionary/reproductive biology of *T. indica* as well. The changes in existing environmental conditions (elevated temperatures, changes in rainfall pattern, drought and elevated CO_2 levels) in areas where Karnal bunt exists are perceived to affect its presence and severity through increased pathogen fecundity. For instance, many climate change models have predicted continuous as well as very heavy precipitation events and these can be favourable for teliospore viability and germination potentially leading to enhanced infection and consequently enhanced disease development probability[13]. There is even the possibility of host range expansion to non-wheat crop species[1,14]. This may have peculiar consequences for wheat as well as new host-based cultivation systems with increased inoculum and accelerated pathogen evolution. There is a chance that the increased CO_2 concentrations and precipitation might render the chemical fungicides ineffective or partially effective[5]. Climate change will make new geographies vulnerable to Karnal bunt, as has been proposed in the case of Europe by Riccioni *et al.*[6]. However, on a positive note, there is a possibility of climate change adversely affecting the recombination process of the heterothallic *T. indica* and in such circumstances, pathotype stabilization can be expected due to low evolution rate leading to relatively easier disease management[5].

After its discovery in 1931, the disease remained little known and confined to northern India until the ushering of the green revolution in the 1960s, when first outbreaks of Karnal bunt were observed in the entire northern India. The main reason for this sudden spread in northern India was that the varieties covering a large area in the country during the early phase of the green revolution, viz. Sonalika, Kalyan Sona, WH147, C306, etc. and also varieties of the late phase of the green revolution, viz. HD2009, WL711, UP262, etc. were not harbouring the genetic resistance for Karnal bunt unlike the native Indian varieties or 'sorts', i.e. the wheat landraces. The native varieties had morphological barriers (pubescence, wax, etc.) which restricted *T. indica* infection and the inoculum build-up remained under check. However, the Mexican high-yielding varieties lacked these barriers and the fungus had the opportunity to cause massive infection that resulted in huge inoculum build-up and consequently the Karnal bunt outbreaks[15]. The large-scale multiplication accelerated the evolution and virulence acquisition of the pathogen. Apart from this, the green revolution period witnessed global movement of seeds carrying the *T. indica* inoculum into many countries where it was not present earlier. At present, Karnal bunt has confirmed presence in Iran, Iraq, Nepal, Pakistan (Punjab and North-West Frontier Province), South Africa (Northern Cape Province), Mexico (Sonora, Sinaloa and Baja California Sur), USA (New Mexico, Arizona, Texas and California) and Brazil (Rio Grade do Sul)[5]. It was only in the 1990s that the importance of placing *T. indica* under a set of quarantine regulations to check its further spread to the new geographies was understood and eventually evaluation of the existing gene pool for resistance against it was begun.

Strides in the development of management protocols for Karnal bunt

Karnal bunt is a seed (Figure 1), soil and airborne disease and consequently the management protocols pertain to checking the pathogen infection from these routes. The use of Karnal bunt free seed needs to be taken into account first for raising a healthy crop. For reducing inoculum load in the infected soil, adoption of a five-year crop cycle (non-wheat crops in the winter season) has been recommended based on the five years survivability period of infection causing teliospores in the soil[16]. The soil inoculum can also be reduced by an increase in temperature by mulching the soil with polythene or even with deep ploughing. As the February rains, coinciding with the heading stage of wheat crop, make it vulnerable to *T. indica* infection, optimization of sowing time becomes crucial for managing Karnal bunt through a successful disease escape. Chemical fungicides have also been reported to be highly effective in reducing the disease incidence and intensity, although 100% salvation is not possible. Propiconazole (0.1%), triadimephone (0.2%), mancozeb (0.25%) and carbendazim (0.1%) are the molecules found effective when any of them is sprayed twice, first at the flowering stage followed by the second after two weeks[8]. *Trichoderma viride* (5 g/l) has been reported to be an effective

Figure 1. ***a***, Healthy grains. ***b***, Karnal bunt-infected wheat grains.

biological control agent for reducing the inoculum load in soil. However, the easiest and also economically and environmentally sustainable method of curbing the Karnal bunt infection is through the deployment of resistant wheat varieties. However identification of durable and diverse genetic resistance and its introgression into high-yielding varieties is time- and resource-intensive with moderate success because of quantitative inheritance with minor genes imparting incomplete resistance for which selection in segregating populations is difficult.

Major advances made in Karnal bunt resistance germplasm development

The breeding for introgression of Karnal bunt resistance in high-yielding wheat varieties was started at the International Centre for Maize and Wheat Improvement (CIMMYT), Mexico in the early 1980s. The annual Karnal bunt screening nursery was constituted from the resistance sources identified from India, China, Brazil and synthetic hybrid wheat[17]. At present, many confirmed sources of Karnal bunt resistance are available in both bread wheat (HD29, HD30, W485, W1786, KBRL10, KBRL13, KBRL22, ML1194, WL3093, WL3203, WL3526, WL3534, HP1531 and ISD227-5) and durum wheat (D482, D873, D879 and D895). Currently, the Karnal bunt resistant cultivars available internationally include Arivechi M92, Navojoa M2007 and INIFAP M97 of bread wheat and Altar C84, Jupare C2001, Aconchi C89, Atil C2000 and Banamichi C2004 of durum wheat[8,18]. The Indian Wheat Programme has developed many Karnal bunt resistant varieties which are recommended for different wheat-growing regions of the country. Among these, HD4672, DWH5023, WL1562, WH1097, WH1100, HDP1731, Raj1555, PBW502, PBW343, WH542 and KRL283 are prominent with different secondary yield attributes. The seven resistant stocks, viz. ALDAN‘S’/IAS58, CMH 77.308, H 567.71/3*PAR, HD 29, HP 1531, W485 and KBRL57 are well established and routinely utilized for introgression in hexaploid background[19,20]. Each year, Karnal bunt resistant lines are identified in the Advance Varietal Trials (AVT) of the All India Wheat and Barley Improvement Programme in different locations and front-line wheat varieties recommended for different zones have been found to harbour Karnal bunt resistance under these trials. In 2019, resistant lines identified included DBW252, DBW273, DBW14, DBW173, DBW187, DBW187, DBW301, DBW304, DBW93, DPW621-50, HD3277, HD3293, HD3345B, HD2932, HD3059, HD3086, HD3226, HD3298, HI1544, HI1612, HI1634, HS507, HS673, K1317, KRL210, MACS6696, MACS5052, MACS6222, MACS6222, MP3336, NIAW 3170, PBW 781, PBW822B, PBW823B, PBW752, PBW757, PBW820, PBW821, PBW824, UAS3002, UAS3002, UP3041, UP3043, VL3019, VL3021, WH1239, WH1080, WH1124 and WH1142. New genetic stocks are also being identified for inclusion in the breeding programmes. One such example is HI8774 which is resistant to yellow rust, powdery mildew as well as to Karnal bunt. The development of Karnal bunt-resistant varieties has proved difficult mainly due to limited variability of genetic resistance against it in hexaploid wheat, quantitative nature of inheritance and influence of the environment on screening for disease resistance leading to limited success in Karnal bunt resistance breeding over the years[5,21]. Therefore, the identification, mapping and tagging of Karnal bunt-resistance genes in wheat is important for developing resistant wheat cultivars.

There is another important aspect of Karnal bunt research and development which is more geo-political in nature. It is the effort of getting the Karnal bunt pathogen de-regularized of the international quarantine regulations. This is mainly led by USA, which has suffered on the economic front because of these regulations restricting wheat exports to Karnal bunt-free countries. Till now, Taiwan, Indonesia, Honduras, Vietnam and Uruguay have deregulated *T. indica* from their quarantine list on the request of the United States Department of Agriculture. The success and failure of such efforts will definitely affect the future course of action as far as the research and development of Karnal bunt-resistant wheat varieties is concerned and with a record production of 107.06 million tonnes of wheat, India needs to have a close watch on such developments.

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Erratum

The murky origins of the coronavirus SARS-CoV-2, the causative agent of the COVID-19 pandemic

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Figure 2 corrected to provide the correct template of SARS-CoV-2 sequence accession number and the correct residue numbering.

Coronavirus/seq no(SARS-CoV-2)	671										681					686			
Bat RmYN01(AGC74176.1)	C	A	S	Y	H	T	A	S	-	L	L	-	-	-	-	R	N	T	G	Q
Bat RaTG13(QHR63300.2)	C	A	S	Y	Q	T	Q	T	N	S	-	-	-	-	-	R	S	V	A	S
SARS-CoV-2(P0DTC2.1)	C	A	S	Y	Q	T	Q	T	N	S	-	P	R	R	A	R	S	V	A	S
SARS-CoV-1(P59594)	C	A	S	Y	H	T	V	S	-	L	L	-	-	-	-	R	S	T	S	Q
MERS(K9N5Q8)	C	A	L	P	D	T	P	S	T	L	T	P	R	S	V	R	S	V	P	G
Human 229E(P15423)	C	A	D	G	S	I	I	A	V	Q	-	P	R	N	V	-	S	Y	D	S
Human NL63(Q6Q1S2)	C	A	D	G	S	L	I	P	V	R	-	P	R	N	-	S	S	-	D	N
Human HKU1(Q0ZME7)	C	I	D	Y	A	L	P	S	S	-	-	R	R	K	R	R	G	I	S	S
Human OC43(P36334)	C	V	D	Y	S	K	N	-	-	-	-	R	R	S	R	G	A	I	T	T

Figure 2. Comparison of the spike protein segment containing the furin cleavage site across viruses specific for bat and human hosts. The top two rows are bat sequences. The middle three are the agents of severe human disease. The last four rows are sequences from the coronaviruses generally causing relatively mild respiratory infections. The blue colour rows highlight the identity of the segments flanking the furin cleavage site in the virus responsible for COVID-19 and a bat virus. Residues conserved at the furin cleavage site (681–686) are highlighted in yellow.