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REVIEW ARTICLE

Tilletia indica: biology, variability, detection, genomics and future perspective

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Abstract
India's wheat production has reached 107.19 million tonnes during 2019–2020. Now country is in a position to export wheat to other countries. However, Karnal bunt disease is a major constraint in wheat export and causes huge monetary loss to wheat trade. The disease is caused by a typical basidiomycetes fungus *Tilletia indica*. It is an important quarantined fungus which affects wheat trade and causes economic loss under changing climatic scenarios. The pathogen was first reported from India then intercepted in other countries. Currently, 77 countries have restricted the import of wheat from the areas where the disease occurs. Recently, the disease has become more severe in the north-western plains zone of India. The pathogen is seed, soil and air-borne in nature. The pathogen has complex genetics, its genetic recombination or mating behaviour between two compatible allantoid sporidia just before infection. We illustrated briefly the complex biology of the pathogen, high genetic variability, detection, genomics, and pathogenesis-related genes in *T. indica*.

Keywords Karnal bunt · Biology · Variability · Detection · Genomics · Wheat · *Tilletia indica*

Introduction

Wheat is the most important cereal food crop and serves as a staple food for millions in the world. The taxonomic position of wheat is family Poaceae (Gramineae) which also includes major food crop such as rice (*Oryza sativa* L.), wheat (*Triticum* spp. L.), maize (*Zea mays L.*), barley (*Hordeum vulgare L.*), rye (*Secale cereale L.*) and oat (*Avena sativa L.*). Wheat belongs to the tribe Triticeae which contain more than 15 genera and 300 species across the world. In 1918, Sakamura (1918) classified wheat into three groups with chromosome numbers, diploids of 2n = 14, tetraploids of 2n = 28, and hexaploids of 2n = 42. In India, wheat cultivation started to date back in the Mohenjo-daro period of about 5000 years ago. Likewise, other crops, several diseases like rusts, Karnal bunt and spot blotch etc. are causing disease in wheat. Karnal bunt is the most important quarantine disease of wheat. Strict quarantine is imposed by many countries to prevent its introduction. It is a major biosecurity concern to exporting countries (Tan et al. 2013; Kumar et al. 2020; Bishnoi et al. 2020). Karnal bunt pose problem in India's participation in the international wheat trade. With reports of as low as 0.01–1 % annual yield loss, it is considered as economic significance of Karnal bunt disease (Saharan et al. 2016). In recent years, grain quality is affected due to re-emergence of the disease in north-western plains zone of India (Gurjar et al. 2016) but, Madhya Pradesh, southern Rajasthan, Maharashtra and Peninsular India are disease free areas in India. Globally, the disease poses an economic threat to the wheat industry due to reduction in grain quality due to production of trimethylamine and yield. Better understanding of mechanism(s) of pathogenesis is required for effective management of disease. This review provides recent advances in understandings the pathogenic and genetic variability, biology, detection, genomics and future perspective.

Karnal bunt of wheat: history and nomenclature

The first report of Karnal bunt was in 1931 from the wheat cultivar Federation and Punjab 8A at Botanical Station of IARI, in the district Karnal of Haryana, India (Mitra 1931).

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The symptom is the development of sori in the ovary with brown to black dusty spore masses of ovoid or oblong shape with 1–3 mm, which attack from the hilum region of grain and continue along the groove of the grain. The disease is also known as partial bunt, stinking smut, new bunt, incomplete bunt and Indian bunt. Till the end of 1970, the disease was of less importance, but later on, severe epidemics of Karnal bunt occurred in India due to the cultivation of high-yielding semi-dwarf varieties, irrigated cultivation, higher doses of fertilizers, and other factors. The host range of Karnal bunt was found to be wheat, durum wheat, rye and triticale (wheat × rye hybrid) (Singh et al. 1989).

The disease has entered Canada and the United States from Mexico (Boratynaski et al. 1985). After that, the disease has been reported in California (Ykema et al. 1996). Karnal bunt was existed in Arizona in 1992 because the wheat samples of 1992 were preserved by the Arizona Department of Agriculture, were tested and teliospores of *T. Indica* was found in grains harvested in 1993. Ykema et al. (1996) also found that infected wheat grains were shipped to New Mexico and Texas and were grown there, causing spread of *T. indica* (Mitra) (synonym *Neovossia indica* Mundkur). The name "smut" was given due to its dusty and black and brown teliospores (Bonde et al. 1997). The class name Ustilaginomycetes was originated from "ustulatus" which means "burned" (Carris et al. 2006). *Tilletia* that infect cereal food crop which produces teliospores in the ovaries of their host plants are referred to as bunt fungi, also considered to derive from the burned word (Duran and Fischer 1961). The name *Tilletia indica* was given by Mitra (1931). Later the pathogen was kept in the genus *Nevossia* due to the production of a large number of primary sporidia which is of unfused. This transfer of genus was supported by Krishna and Singh (1982) suggested that *N. Indica* is closer to *Neovossia* genus than to the genus *Tilletia*. But later in 1953, Fischer (1953) agreed with Mitra's generic name and disagreed with Mundkur's point of view and again referred to the pathogen as *T. indica*. The name *Tilletia indica* also accepted by The Commonwealth Mycological Institute because the fragmentary appendages present on teliospores and unfused sporidia are not typical of *N. Indica* emphasized by Mundkur, but also basic features of other *Tilletia* species. And now taxonomic question persists and in the literature, *T. indica* is considered a synonym of *N. Indica.*

Prevalence of Karnal bunt across the world

The disease has been reported in other countries like Afghanistan, Pakistan, Nepal (Munjal 1975; Singh et al. 1989), Mexico and in some parts of the United States (Duran and Cromarty 1977), Iraq (CMI 1974), Iran (Torabi et al. 1996), Lebanon, Syria, Sweden and Turkey (Lambat et al. 1983), Brazil (Da Luz et al. 1993) United States (Ykema et al. 1996) and South Africa (Crous et al. 2001). The pathogen occurred as widespread in 'Northern' and 'Central India' viz.. Delhi, Haryana, Uttar Pradesh, Himachal Pradesh, Punjab, Rajasthan, Jammu and Kashmir, Madhya Pradesh, West Bengal, and Gujarat where high humidity and low winter temperatures are prevalent (Singh et al. 1985). Grain yield loss of 0.2 % based on the total wheat production in North India assessed which is found to be equivalent to forty thousand metric tons of wheat grain per annum (Munjal and Chatrath 1976). A loss of 0.2–0.5 % of the total wheat production occurs in India due to Karnal bunt (Joshi et al. 1983), 2–3 % loss in the total wheat grain production in Pakistan (Hassan 1973). Whereas, in Mexico losses due to Karnal Bunt was found to be 0.3–2.1 % of total wheat production across several states in Mexico (Fuentes- Davila 1996). The state of the various important grain markets of Punjab and India was spotlighted by Sharma et al. (2004). They could find that there was variation in disease each year, which was evident from the percentage of diseases free samples recorded in each year. Karnal bunt is emerging in some parts of India, Iran, Iraq, Mexico, Nepal, Pakistan, South Africa and USA (Jones 2007). Based on the information, the high risk of establishing Karnal bunt in Europe was assessed (Jones, 2009). Since then, the disease has occurred frequently in the north-western plains of India (Praveen et al. 2015, Gurjar et al. 2016).

Symptoms, pathogen and disease cycle

One of the major difficulties in the detection of Karnal bunt in field conditions is that the symptoms are not much visible, which make delay in diagnosis and further management of the disease. The disease is also called partial bunt due to its characteristic symptom of bunt sori formation only on few grains in the head and not all the heads of the wheat plant is infected also a portion of the wheat grain is infected, which make Karnal bunt symptom differ from other bunt diseases of wheat. Infection is mainly confined to endosperm and ventral groove of the kernel. In field conditions, silvery black shiny spikelet of the infected kernel with swollen ovaries and glumes spread from each other. Another important symptom associated with Karnal bunt is that the infected grains emit a rotten fishy smell due to the production of chemical trimethylamine. Seed- or soil-borne teliospores and their successive germination seem to play only a starting role in Karnal bunt epidemics.

The pathogen moves systemically within the head through the rachis and causes infection to the kernels (Dhaliwal et al. 1983). But one year later studies, showed that spread of *T. indica* from the infected spikelets to another spikelet of the same spike found to be aerially rather than systematically

within the ear of wheat (Bedi and Dhiman 1984). This conclusion was made from their observation that there was no presence of diseases causing mycelia portion in the rachis bearing infected spikelet, also found simultaneous infection of kernels in the spikelet. Teliospores of *T. indica* has a diameter of 22–49 µm with an average of 35 µm at maturity and are diploid, with globular to sub-globular in shape also. Spores of *T. indica* can resist adverse environmental conditions. It can be viable up to 2–5 years in the soil (Mathur and Cunfer 1993).

Newly formed teliospores have dormancy that can be broken by exposing them to high temperatures of 40–43 °C for 18 days or more under direct sunlight. Soaking teliospores in peptone, wheat straw extract, benzaldehyde, furaldehyde, or butyric acid can affect dormancy and in plain water, only 50 % of teliospores germinate. They retain germinability for 2 years if teliospores are buried deep in the topsoil. By various treatments like keeping teliospores at 15–20 °C for 10–15 days can break the dormancy and allow the spores to germinate. Stout promycelium develops from the germinated teliospores, and it bears 60–185 primary sporidia clusters at the tip. The primary sporidia are short-lived and sensitive which can germinate in water and produce a thick mycelial mat. From the mycelia mat, crescent-shaped secondary allantoid sporidia will be produced. Occasionally, secondary sporidia exhibit yeast-like tendencies to bud and develop another crop of allantoid spores on a wet leaf surface. The pathogen follows different pathways to produce spore, depending on the availability of free water and temperature was known (Singh et al. 1996; Dhaliwal and Singh, 1988; Dhaliwal et al. 1989 and Smilanick et al. 1989). There are three distinct layers in the teliospore which can be observed through scanning electron microscopic. The layers are outer perisporium, middle episporium and inner endosporium (Aggarwal et al. 1998). Inspection of the infected kernel has shown that the pathogen disintegrates the parenchymatous tissues in the pericarp and the hyphae proliferate there and produce teliospore, later the hyphae direct towards the base of glume and enter into sub-ovarian tissue and then it direct towards and enter into the pericarp of seed through funiculus (Goates 1988). The embryo of the seed is not infected in normal environments, even under severe conditions; the embryo is free from infection (Aggarwal et al. 1994).

During the process of germination, the diploid nucleus of the spore undergoes meiosis which will be followed by several cycles of mitotic divisions. Initially, pinhead size promycelium emerges from the teliospore which continues to elongate. At the distal end of promycelium, 180–185 haploid primary sporidia (basidiospore) are produced (Bansal et al. 1984; Smilanick et al. 1985). In normal condition, teliospores which are germinating at a depth of 2 mm of soil are impotent to outstretch to the surface. Primary sporidia have a length of 64–79 µm and a width of 1.6 and 1.8 µm. Primary sporidia germinate to turn out to mycelia, which subsequently makes a large number of infectious secondary sporidia. Secondary sporidia are of two shapes (allantoid and filiform) in which the former one is more infectious. The size of secondary sporidia varies for diverse isolates from 11.9 to 13.0 µm with an average width of 2.0 µm (Peterson et al. 1984). The dispersal of pores is probably during flowering, during which the primary and secondary sporidia are probably splashed and gusted onto the glume surface which is present around the wheat grains (Peterson et al. 1984).

Research undertaken by Dhaliwal and Singh (1988) unveiled that *T. indica* can progress from the soiled facet to susceptible ear heads. Their study has revealed that the sporidia present on the soiled facet germinate on the leaves of the lower plants, and populate the surface of the leaves which in turn produce a large number of sporidia which are splashed or blown to the leaves which are present in the upper portion of the plant. In this fashion, the infectious spores progressed to the top of the plant ultimately reach the developing heads. Once it reaches the developing head, sporidia on the glumes germinate and take root through the stomata when the plant is in the sensitive period of 2–3 weeks at the anthesis or near anthesis. The mycelia advance to the base of glumes and also to the growing grains. The fungus restricts intercellular in the pericarp. By the time grain, mature the spores proliferate inside and produce a mass of black teliospores. During harvesting time, the spores are re-thrown down to the soil surface to carry over the pathogen and thus disease. A higher number of teliospores detected ranging from 1762 to 368,332 teliospores in Karnal soils using qPCR assay (Gurjar et al. 2017a).

Host range and quality of grains

The natural host for Karnal bunt pathogen is common bread wheat (*Triticum aestivum*) from which the pathogen was first time isolated by Mitra (1931). Another important host is durum wheat (*T. durum*) and the next important host for Karnal bunt pathogen is triticale (*Triticosecale*) (Agarwal et al. 1977). *Tilletia Indica* can infect *Triticum variabilis, Tritium shareonensis, Triticum scerrit* and *Triticum ovatum.* Even though, artificial inoculation of *T. indica* produces disease in the accessions of *Oryzopsis miliacea, Lolium multiflorum, L. perenne, Bromus ciliates, Bromus. tectorum, Triticum monoccocum, T. trimopheevi, Aegilops triuncialis, Ae. Columnaries*, *Ae. mutica*, *Ae. caudate*, *Ae. squarrosa*, *Ae. sharonensis*, *Ae. cylindrica*, *Ae. comosa Ae. bicornis*, *Ae. triaristata Ae. searri* (Aujla et al. 1985). Infections under the natural conditions, these plants are not yet known (Royer et al. 1986; Royer and Rytter, 1988).

The main economic impact of Karnal bunt disease is that it affects majorly the quality of grain rather than the

quantity of the grain. It decreases the viability and weight of seed and also worsens the flour quality by the production of fishy-smelling trimethylamine (Singh et al. 1983). Studies undertaken by Mehdi et al. (1973) have reported that when the grains are infected 3 % or above will effuse fishy foul odour and it will make the grains unpalatable for consumption. Hussain et al. (1998) suggested grains with 1–4 % of infection can be consumed by blending with healthy kernels. Investigation on the quality parameters of infected grains (Bhat et al. 1980) revealed the amount of trimethylamine can be reduced by the washing of grain followed by drying so that a load of chemicals was reduced because of its volatile nature. It was shown that feeding of weanling rats with 50 % bunted kernels for 45 days does not show any adverse effect. Toxicological studies conducted in monkeys also confirmed that consumption of grains up to 70 % infection was not toxic to animals. Infected bunt seeds were used for feeding animals in Mexico (Brennan et al. 1990). In India, many families rejected infected grains beyond 2 % infection (Nagarajan 1997). As the teliospores of *T. indica* cannot persist in the process of milling, the infected kernels have to be milled to produce flour along with healthy grains. Such products can be converted to various alternate forms like flakes. It is also found that the viability of teliospores can be reduced by producing animal feeds using steam flake milling and Holo flite thermal processing (Bonde et al. 1997).

Pathogenic and molecular variability in *Tilletia indica*

Variability in number and size of chromosomes was observed in *T. indica* with at least 11 chromosomes ranging from approximately 1–3.3 Mb (Tooley et al. 1995). Many isolates had peculiar karyotypes. Variations in teliospore karyotype and mono-sporidial lines derived from the same teliospore suggested that changes in karyotype occur in meiosis (Tooley et al. 1995). Race existence in *T. indica* is controversial (Gill et al. 1993). Based on different levels of aggressiveness four races have been defined (Bonde et al. 1996). From this study, it was concluded that there was no evidence in nature regarding races. *T. indica* heterothallism is the most possible cause of the regular reassortment of the gene, resulting in race instability. Wheat showed substantial variations in the degree of susceptibility among cultivars. *T. indica* also showed a high level of genetic variability among the isolates and display a diverse level of virulence (Mishra et al. 2001).

A thorough understanding of the molecular mechanism of variability generation is prerequisite for the development of resistant wheat varieties with a wide genetic base. According to Rai et al. (2000) as the pathogen is hemi-biotrophic the possible source of variability generation is through recombination between compatible mating types. Another possibility of variability generation is given by Nicholas (1998), through anastomosis followed by parasexual recombination or even by a simple mutation. The use of genetic markers has played an important role in understanding the ambiguity in the genetic variability. DNA polymorphism has been generated by using commonly used genetic markers like restricted fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs) which all helps to detect the genetic and phylogenetic relationship (Majer et al. 1996; Milbourne et al. 1997; Pimentel et al. 1998; Mishra et al. 2001; Powell et al. 1996; Levy et al. 2001; Castlebury et al. 2005; Singh and Gogoi 2011; Thirumalaisamy et al. 2012).

Primary and secondary sporidia as compatible mating types fuse to form dikaryon thus increase the chances of variation (Dhaliwal and Singh 1988). In earlier studies, the genetic and pathogenic nature of the fungus was found variable due to recombination of secondary allantoid sporidia (Aggarwal et al. 2010). Earlier Duran and Cromaty (1977) have also reported that *T. indica* being a heterothallic fungus demands fusion between secondary sporidia of opposite mating types resulting in high variation. A study conducted by Thirumalaisamy et al. (2006) demonstrated the pathogenic variability in *T. indica.* All the collected isolates showed a pathogenic variation on eight host differentials. Isolates from Gurdaspur (KB5), Kapurthala (KB6), Faridkot (KB7), Sangrur (KB8), Bareilly (KB15) and Hardoi (KB16) were compared their aggressiveness with KB17 and were classified as KB-Ag (I) Narwana (KB1), Karnal (KB2), Samalkha (KB4), Ambala (KB13), Hoshiarpur (KB10), Ludhiana (KB9), Hapur (KB3), Pantnagar (KB12) and Delhi (KB14) isolates were grouped under KB-Ag (II) Four isolates of least aggressiveness belonging to Jammu (KB18), Bilaspur (KB11), Solan (KB20) and Almora (KB19) were grouped as KB-Ag (III) For the evaluation of genetic relatedness within species, a new DNA typing tool named MLST, Multilocus sequence typing has developed in *T. indica* (Gurjar et al. 2018a). This approach is focused on DNA sequence analysis of nucleotide polymorphisms in the housekeeping gene for intra-specific differentiation in bacterial and fungal pathogens. To separate these *T. indica* isolates seven multilocus sequence fragments were chosen. A phylogenetic tree was developed based on combined phosphoglycerate kinase (PGK), actin-related protein 2 (ARP2), beta-tubulin (TUB), glyceraldehyde-3-phosphate dehydrogenase (GAPDH eukaryotic translation initiation factor 3 subunit A (EIF3A) and serine/threonine-protein kinase (STPK), histone 2B (H2B), together with the reference sequences. The phylogenetic tree developed has grouped the isolates into two major clusters. In cluster I, reference gene was included. Isolate KB-11 from Kaithal, Haryana was found to be highly conserved as it

was positioned under cluster I which was having maximum sequence similarity with the reference isolates. KB16 and KB17 from Uttar Pradesh and KB 19 from Haryana are the other isolates under cluster I. Under cluster II, most diverse isolates like KB-18 (Muzaffarnagar, Uttar Pradesh) KB-07 (Jind, Haryana) were grouped. They could also identify single nucleotide polymorphism from the amplified gene sequences. The maximum number of single-nucleotide polymorphisms of 675 was for the gene Phosphoglycerate kinase. Whereas the minimum number of SNP was found in KB-11 isolate from Kaithal, Haryana. The highest number of SNPs of 165 was found in KB-18 and 164 in KB-14 from Sultanpur, Uttar Pradesh. The most diverse of all indica isolates were found to be KB-18 isolate (Gurjar et al. 2021).

Another study (Gurjar et al. 2016) revealed that Karnal bunt isolates have been grouped into two major phylogenetic tree clades. The seventeen isolates were viz.. KB1, KB7, KB11, KB15, KB12, KB16, KB19, KB3, KB2, KB10, KB9, KB17, KB20, KB18, KB2, KB6, KB13 clustered in clade I and remaining four isolates viz.. KB5, KB4, KB8, KB14 clustered in clade II with an outlier of *T. horrida* and *Ustilago maydis*. Among isolates, 5 isolates of KB1 from Bareilly, Uttar Pradesh, KB12 isolate from Bulandshar, Uttar Pradesh, isolate KB7 from Aligarh, Uttar Pradesh, isolate KB11 from Bulandshar, Uttar Pradesh and isolate KB15 from Kota, Rajasthan matched 100 percent in subgroup I of clade I and two isolates KB18, KB21 were also similar in subgroup II of clade I. Some isolates showed nucleotide variation which ranged from one to two and the majority of the isolates did not cluster region-wise; from this study, it is evident that isolates showed a high genetic variation at the molecular level in a single KB seed.

Epidemiology

Karnal bunt disease usually occurs sporadically and the prevailing weather and the change in wheat varieties have significantly affected the occurrence of Karnal bunt, so the onset and severity of the disease differ over the years. Duration for soaking teliospores in water is directly related to the germination of spore. Incubating teliospores at 30 °C for about one week followed by treating spore at 18 °C for another one week was found to positively help the germination (Bansal et al. 1985). Seed and soil-borne inoculum has found to cause 0.76 and 0.31 percent infection respectively.

Treatment of spores with liquid nitrogen has been found to enhance germination (Bansal et al. 1984). Effect of different temperature on teliospores germination was conducted by Kumar et al. 2007). It revealed that at − 5 °C teliospores produce ruptured basidium, at 10 °C few filiform sporidia formed, proper basidium and the full number of filiform sporidia were produced at 15–25 °C. The development of abnormal germ tube when incubated in extreme temperature conditions viz. low temperatures of − 5, 5, and 10 °C, high temperatures of 35 and 40 °C. It was found that the optimum temperature for the production of normal germ tube was found to be 15, 20, 25 and 30 °C. And maximum production of sporidia was found to be between 20 and 25 °C (Kumar et al. 2003). With the increase in depth of soil the viability and density of spores were found to be reduced. Studies from different soil of Dhaulakuan district of Himachal Pradesh have shown that up to 6 cm the concentration of viable spores are high. Teliospores viability can be reduced by thawing snowing and chilling which can prolong the germination period, which indicated that the occurrence of disease in the place where snow occurs will be very infrequent (Sidhartha et al. 1995).

Under high relative humidity and leaf wetness during 2.0–6.0 AM the release of secondary sporidia was found to be highest (Sidhartha et al. 1995). They also showed that wind speed and solar radiation adversely affect the sporidial release. The sporidia count coincided with the anthesis cycle of the crop during the last week of February to the first week of March. A temperature of 15 °C before inoculation predisposes the infection and for spread, an optimum post inoculation temperature of 18 °C was found to be effective (Sidhartha et al. 1995). Teliospores attain maximum viability after a maturity period of 22 weeks (Schall 1991). Research carried out by Babadoost et al. (2004) in the Northern United States showed the recovery of teliospores from soil sample was 90.2, 18.7.16.1 and 13.3 % after the introduction of teliospores into the soil at different time points (1 day, 8, 20 and 32 months). The effect of spray inoculation of sporidia during various stages of wheat growth was studied and it was found the most susceptible stage for infection as awns tip-out stage (Aujla et al. 1989).

The disease was developed in one occasion if a higher number of soil-borne teliospores and a conducive environment persists. Further, there was not direct relationship was found between soil borne teliospores and disease occurrence (Allen et al. 2009). The disease prediction based on the Humid Thermal Index (HTI) model revealed that *T. indica* can establish in sixty percent of locates in China (nearly 4,580,000 Km^2) (Wei-chuan and Gui-ming, 2010). Weather data of 1st to 12th standard meteorological week (1st January to 25th March) during 1981–1982 to 2004–2005 at Karnal station, Haryana, India were analysed. It suggested that meteorological parameters during the 6th to 12th SMW suitably clarified the occurrence of the pathogen (Singh et al. 2010a, b). Airborne dispersal of teliospores of *Tilletia* sp. in grain warehouses is required much attention to check further spread of inocula (Halasz et al. 2013). The seed material should be checked to further spread of the spores. There is the need to find out the relationship between

disease occurrence and soil-borne teliosporic population in hot spot areas of the pathogen.

Detection and diagnosis of Karnal bunt

A simple method was developed for the detection of Karnal bunt by soaking seed samples in 0.2 percent NaOH solution for 24 h at 20 °C (Agarwal and Verma 1983). Castro et al. (1994) has demonstrated a filter centrifuge technique for the extraction of spores from the infected grains. Morphological characterization was done under SEM and it revealed several surface ornamentation differences in teliospores (Castlenbury 1997; Aggarwal et al. 1998). Even though, microscopy-based morphological detection is not much accurate and reliable.

Protein profiling through SDS PAGE was done by Kutilek et al. (2001) and revealed a comparison between *T. indica* with *T. tritici T. barclayana T. laevis and T. controversa.* Double antibody sandwich ELISA and dipstick immunoassay were carried out by using antibodies developed against a 60 KDa protein of *T. indica*. But differentiation of *T.indica* from other species of *Tilletia* was not successful by using the serological method. For the identification of quarantined and non-quarantined *Tilletia* species, Mishra et al. (2002) have developed the RAPD-PCR technique. Based on the amplification of ITS region Levy et al in 2001 have carried out RFLP-PCR to distinguish *T. walkeri* and *T. indica.* Ferreira et al. (1996) has developed a protocol for the isolation of species-specific mitochondrial-RNA from *T. indica.* Frederick et al. (2000) developed five sets of PCR primers from 2.3 Kb mt-DNA sequence, specific to *T. indica* and three sets specific for *T. walkeri* based on single nucleotide difference at the 3' end of forward and reverse primers. However, amplification of a single ungerminated teliospore was not achieved, which is essential for Karnal bunt free wheat trade. A nano immunosensor was developed for spore detection of *T. indica* (Singh et al. 2013).

A real-time PCR-based diagnostic marker has been developed for the quantification and detection of teliospores from the soil (Gurjar et al. 2017). In this study, teliospores collected from Karnal and IARI farm soils by centrifugation method were 450 and 1341 respectively. The qPCR method a gave higher number of teliospores ranging from 1762 to 368,332. This diagnostic marker developed could be used to detect teliospores accurately, reliably and quickly in soil. The real-time molecular protocols were reported to detect 3–5 spores in grain washing (Tan et al. 2009) and the protocol was enhanced to detect *T. indica* (Tan et al. 2010). The LAMP assay was developed to detect the *T. indica* (Tan et al. 2016). A multiplex PCR-based marker was validated in *Tilletia* sp. (Valente et al. 2019). Rapid and specific detection based on loop-mediated isothermal DNA amplification assay was developed to detect *T. indica* (Gao et al. 2016). A patent was made for methods, reagents, and kits for detection of Karnal bunt (Tan 2020). Kumar et al. (2008) elaborated the molecular and immuno-diagnostic tools for detection, surveillance and quarantine regulation of *T. indica*. Nano-immunosensor for fungal spore antigen of *T. indica* developed (Singh et al. 2013). An immunodiagnostic assay was developed for the detection of Karnal bunt (Singh et al. 2015). An attempt was made to develop a surface plasmon resonance-based immunosensor for Karnal bunt diagnosis (Singh et al. 2010a, b). Recently, an immunobiosensor designed and developed for early detection of Karnal bunt of wheat (Mishra et al. 2020).

Host resistance and genetics

The sources of genetic resistance for KB are now available in both bread wheat (HD 29, HD 30, W 485, W 1786, KBRL 10, KBRL 13, KBRL 22, ML 1194, WL 3093, WL 3203, WL 3526, WL 3534, HP 1531, ISD 227-5) and durum wheat (D 482, D 873, D 879, D 895) for breeding purpose. Resistance has been incorporated in high yielding wheat varieties like PBW 343 and WH 542 by backcrossing (Sharma et al. 2016a, b; Brar et al. 2018) Although, the development of KB-resistant varieties has proved difficult mainly due to limited variability of genetic resistance against KB in hexaploid wheat, quantitative nature of inheritance and influence of environment on screening for disease resistance (Dhaliwal and Singh 1997; Chhuneja et al. 2008) leading to limited success in KB resistance breeding over the years (Kaur et al. 2016). The dikaryotization of compatible mating types just before the infection causes pathogen genetic recombination resulting in reduced disease incidence and high frequency of escapes even with artificial inoculation leading to confounded outcomes of the screening experiments (Dhaliwal and Singh 1997). The development of molecular markers closely linked to resistance QTLs for trait selection and eventual gene pyramiding can help in the selection of resistant genotypes in the absence of pathogens (Singh et al. 2007). Therefore, the identification, mapping and tagging of KB-resistance genes in wheat is important for developing resistant wheat cultivars (Kaur et al. 2016). A multitude of resistance sources have been identified lately, however, due to a lack of genetic analysis of these sources, their utilization in the development of resistant cultivars has not been very successful (Brar et al. 2018). The mechanism of host-pathogen interaction leading to a successful infection remains ununderstood making the cultivar development even more challenging.

Sirari et al. (2008) have carried out studies in the genetics of resistance of wheat against Karnal bunt. They noted that the accuracy of genetic analysis of resistance diminishes

when they use a heterogeneous mixture of inoculum for Karnal bunt screening. A cross between susceptible parent WL711 and resistant parent HD29 has been done to develop a set of 130 recombinant inbred lines, which were further utilized for screening for 3 years with *T. indica* prevalent in North India. When mapped 81 AFLP and 90 SSRs on the recombinant inbred line, it could find chromosome markers 2A, 4B and 7B collectively accounted for around one-third of disease reaction variations. The 4B chromosome's long arm has the largest effect which reduced Karnal bunt occurrence by half which was evident from three separate experiments, which account for up to 25 per cent of the variation in the phenotype for KB reaction. Marker-assisted selection for the resistance of Karnal bunt in can be carried out by GWM538 a closely linked SSR marker (Singh et al. 2003). Genome-wide association mapping of Karnal bunt resistance was studied (Gupta et al. 2019). The complex genetics of Karnal Bunt (*Tilletia indica*) resistance in wheat was studied using genetic linkage and genome-wide association analyses (Emerbiri et al. 2019). The pathogen has complex genetics its genetic recombination or mating behavior between two compatible allantoid sporidia just before infection. The resistance stocks, cultivars and breeding efforts against Karnal bunt disease were described in detail (Bishnoi et al. 2020).

Genomics

In molecular biology, there is essential to understand pathogen evolution, biology, lifestyle, novel disease management using molecular approaches. Whole genome sequence of pathogens has led to marker development, genetic diversity and *in silico* identification of pathogenicity-related genes leading to better management of the diseases. Genomes of two (PSWKBGH_1, PSWKBGH_2) monosporidial lines of *T. indica* was sequenced (Sharma et al. 2016a, b). The genome sequenced with 26.7 Mb, 31.38 Mb assemblies and GC content of 53.99 % (Kumar et al. 2017; Kumar et al. 2018). Recently, the whole genome was sequenced having 33.7 Mb with 55 % of GC (Gurjar et al. 2019) and assembly of genome was near to complete genome of *T. indica*. *Tilletia* species-specific genes were identified based genome sequence of *Tilletia* sp. and validated (Nguyen et al. 2019). Fourteen pathogenicity related genes viz.. *TiHog1*, *TiHsp70*, *TiPmk1*, *TiKss1*, *TiHos2*, *TiChs1*, *TiKpp2*, *TiCts1*, *TiUbc4 and TiUkc1 TiSsp1*, *TiSte20 TiPrf1 and TiSid1* were identified in *T. indica* (Gurjar et al. 2017b, Gurjar et al. 2018).

A set of protein was found to be up-regulated in mycelia of *T. indica* (15 proteins) and secreted proteins (20 proteins) in the proteomics analysis. Proteins were found to be associated with host defense suppression, degradation of lignin from the plant cell wall, attachment of pathogen to cell wall, pathogen mediated ROS production, detoxification of plant's ROS, enzyme production etc. (Pandey et al. 2019). Research has been attempted to upgrade the draft genome by following reconciling the globally accessible datasets of three highly virulent monosporidial cultures *T. indica* field isolates. Orthologous genes to basidiomycetes were found to be 79 %, and proteins with secretary signals were nearly 7.93 %, and highly virulent pathogenicity genes were found to be 6.66 % (Mishra et al. 2019). Identified proteins were involved in suppression of host defense responses, lignin degradation of a plant cell wall, penetration, adhesion of pathogen to host tissues, pathogen mediated reactive oxygen species generation, hydrolytic enzymes, detoxification of host generated reactive oxygen species. The putative secretome analysis of *T. indica* genome has revealed the expression of gene *TiHOG1*, which was expressed with a fold change of 19.81 in response to a susceptible host, but showed a lesser expression of 9.65 fold change in case of resistant host. It revealed that the gene *TiHog1* may have role in pathogenesis apart from stress tolerance (Gurjar et al. 2018). Comparative genome analysis of *T. indica* revealed high genomic variation (Gurjar et al. 2020). Singh et al. (2020) also identified few pathogenicity-related genes in *T. indica* which may have role in sporulation, infection and penetration. The functional genomics attempt to illustrate the functions and interactions of genes and proteins in *T. indica*.

Future perspectives

The present climate change scenario, the disease is remerging in north-western plains zone of India. Till now, the pathogen has been reported few countries. Keeping in quarantine importance, the pathogen movement must be restricted to other areas and countries. The pathogen has complex biology need to understand to compatible monosporidial lines of *T. indica* to cause the disease. The mystery of spore dormancy and germination a key question that remains unresolved till now. So far, the mechanism of teliospores germination of *T. indica* and threshold level of teliospores to cause disease is not understood. Any effector (virulence) and R gene combination are not yet known for *Tilletia indica*-wheat interaction. The mating-type genes involved in virulence to cause the disease is needed to be identified in *T. indica*.

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