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Karnal bunt of wheat (*Triticum* spp) — A global scenario

D V SINGH[1] and ROBIN GOGOI[2]

Indian Agricultural Research Institute, New Delhi 110 012

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ABSTRACT

Karnal bunt of wheat was first reported in 1931 from Karnal, India. The pathogen infects the ovary and converts the grain into a sorus of dark coloured teliospores. The losses occur in terms of quality and quarantine reasons. The soil-borne teliospores after germination produce primary and secondary sporidia under ideal conditions. The allantoid secondary sporidia become airborne by wind or rain splash and infect the emerging spikes. The heterothallic and compatible secondary sporidia fuse prior to infection. The infected grains emit a rotting fish smell due to the production of trimethylamine. The cultivation of resistant varieties, mulching of wheat with chickpea or polythene, use of biocontrol agents like *Trichoderma* spp and low doses of systemic fungicides like Tilt (propiconazole) and Folicur forms the major components for the integrated pest management strategies. Karnal bunt of wheat has become a major SPS issue in wake of recent Sanitary and Phytosanitary Agreement stipulated by World Trade Organization. Under this agreement each member country has to undertake pest risk analysis for import or export. In the present article, global scenario of Karnal bunt disease along with its different aspects like distribution, pathogen, epidemiology, detection and management strategies have been reviewed.

Key words: Diagnostics, Epidemiology, Karnal bunt, Management, Pest risk analysis, Quarantine, *Tilletia indica*, Wheat

Wheat (*Triticum aestivum* L. emend. Fiori &Paol.) is an ancient crop in the Indian sub-continent. *Artha-Veda* written in 1500 to 500 BC refers to wheat and in *Rig Veda* existence of two types of wheat varieties (awnes and awnless) has been mentioned. Despite a long history of wheat cultivation in the sub-continent, its yield/unit area had been very low. The wheat production was 10–11 million tonnes in 1965. Situations, however, has changed considerably due to introduction of new Mexican dwarf high-yielding varieties, which required better field management, high input of fertilizers and irrigations and responsible for 'Green Revolution' in the country in mid 1960s' and 70s'. During 2008–09, India produced a record wheat production of 80.58 million tonnes. Despite of record food production, India continues to be under pressure due to high population growth rate and utilization of fertile land for urbanization and industrialization.

One of the major constraints in boosting up the wheat production is the prevalence of a number of fungal diseases. Among the major diseases of wheat, important ones are three rusts- stem, leaf and stripe; loose smut, flag smut, Karnal bunt , hill bunt, foliar blight and powdery mildew (Joshi *et al*. 1986).

Occurrence and distribution

Karnal bunt [*Tilletia indica* (Mitra) = (*Neovossia indica* (Mitra) Mundkur] was first reported by Mitra (1931) from Karnal (Haryana). It was earlier considered to be a disease of minor importance. In 1969–70, the disease appeared in severe form in parts of Punjab, Haryana, Rajasthan and western Uttar Pradesh, but the intensity was very low. Based on survey data over the years, the disease found to be established in Punjab, Haryana, Delhi, Uttar Pradesh, parts of Rajasthan, Madhya Pradesh, Bihar and West Bengal, lower altitudes of Himachal Pradesh and Jammu region of Jammu and Kashmir. The disease was not recorded in Maharashtra, Gujarat, Orissa, Assam, Meghalaya, Karnataka, Andhra Pradesh, Tamil Nadu and Kerela (Joshi *et al.* 1983 Singh 1986, Gill *et al*. 1993). Karnal bunt normally occurs sporadically and greatly influenced by prevailing weather conditions and shift in wheat varieties as a result, the prevalence and severity of infection fluctuate greatly from year to year (Singh *et al*. 1985, 1996). The disease is mainly present in Indian sub-continent including India, Pakistan and Afghanistan. Now it has also been reported from Mexico, Iraq, Iran, USA, Nepal (Singh *et al*. 1998) and South Africa (Crous *et al*. 2001).

[1] Former Head and Emeritus Scientist (e mail: dvsingh44@gmail.com; dvs44@rediffmail.com), [2] Principal Scientist (e mail: r.gogoi@rediffmail.com), Division of Plant Pathology

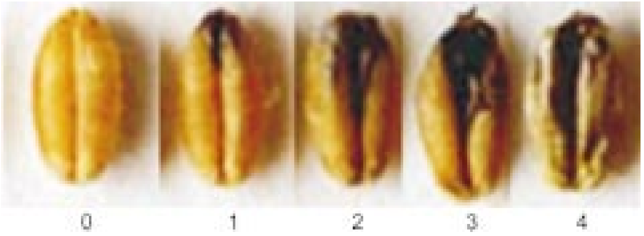

Fig 1 Karnal bunt symptoms - different grades of infection on wheat seed. *0.* Healthy seed of wheat, *1.* Germinal 'Tip' infection, *2.* Infection advancing to the kernel groove, *3.* More advanced infection (3/4th of the grain) and *4.* "Canoe" symptom-the hollowing out of seed interior

Symptomatology

The infection occurs at the flowering stage and the disease becomes evident when grains are developed. In a stool, all the earheads are not infected and in an ear all the grains are not bunted. The fungus affects the grain partially with some tissues of the grain remaining normal and some converted into a mass of bunt spores. However, some individual grains in diseased heads are also completely bunted. In badly infected spikelets, the glumes spread apart and quite often fall off exposing the bunted grains which also fall to the ground. Normally, the embryo tissues except in severe cases is not destroyed. Generally the infection spreads to the tissue along the groove of the grain but the endosperm material lying along the groove of the grain remains uninfected. Different grades of seed infection are presented in Fig 1. Freshly collected infected grains emit foul smell due to the production of trimethylamine (Singh 1986, Gill *et al.* 1993)

Goates (1988) examineed the infection process of *T. indica* into spikes of wheat and found that germ tube arising from sporidia penetrated through stomatal opening of glumes, lamma and palea, but failed to enter sub-stomatal chambers. Generally the endosperm and the dorsal side of the seed remain unaffected. Cashion and Luttrell (1988) have demonstrated that the pathogen does not invade the embryo and the mycelial growth is limited to the pericarp. Transmission electron microscopy shows that the mycelium proliferates in the pericarp by disintegrating the middle layers of parenchymatous cells and prevents the fusion of the outer and inner layers of pericarp with the seed coat. The mycelial mat forms a compact hymenium-like structure and gives rise to short, septate stalks that bear single teliospores.

Economic losses

Karnal bunt is in existence since 1930 in the country but without serious economic losses. The data collected by survey teams show that even during worst years of epidemic, the total damage to wheat crop was only 0.2 to 0.5% of the total production (Joshi *et al*. 1983). Singh (1994) also suggested 0.3–0.5% loss in yield during the most severe years between 1982 and 1989, particularly in Uttar Pradesh.

Brennan *et al*. (1990) estimated direct quality and seed export losses from Karnal bunt to be 0.12%/year in north-western Mexico and estimated the total loss due to quarantine restrictions on Karnal bunt to the tune of 7.02 million US dollar/year. Fuentes-Davila (1998) estimated the losses due to Karnal bunt in three states, namely Sonora, Sinola and South Baja California in Mexico to be 2.1, 2.0 and 0.3% of wheat production, respectively. Thorne *et al*. (2004) conducted a study to assess the economic impact of *T. indica* in the European Union and reported that in case of outbreak of the disease, 50 000 ha of wheat would have significant economic loss due to disruption to production, the inability to export and adopting control measures would impose costs of 454 million Euro in the region over a 10-year period.

The above estimates were merely based on the per cent infected grains mixed with healthy grain lots and do not reflect the pathological effect in the form of sori formation and loss in seed germinability. A direct relationship between sori formation and loss in grain weight and seed viability has been established. Seeds having large bunt sori suffer more loss in grain weight than smaller one (Gill *et al*. 1993, Singh 1994). The survey data between 1984 and 1994 showed that cultivars, like 'Sonalika', 'HD 2329', 'WL 1562' and 'WH 147' occupied maximum area under cultivation, registered low mean infection and their grains had small sori, therefore, the impact of the disease on yield was low. There is little effect of Karnal bunt infection on seed germination. However, badly affected seeds show poor viability or abnormality of seedlings (Singh 1986, Singh *et al*. 1998).

Quality concern

Karnal bunt pathogen not only reduces the weight and impairs the viability of seeds but also causes deterioration of flour quality due to production of trimethylamine (Singh 1986). This fact is also important because flour industry suffers from quality and it poses restrictions in wheat trade. Mehdi *et al*.(1973) examined the effect of bunts on the quality of *chapaties* and attributed that having 3% infection emits a fishy odour and are unpalatable. However, if the grains are washed and steeped, the samples with 7–10% infection are acceptable for consumption (Sekhon *et al.* 1981). It is possible that washing reduces the quantity of trimethylamine which is a volatile substance. No mycotoxins have been detected in Karnal bunt infected grains. Tests for ergot alkaloids were also negative. Short-term toxicity studies in rats by feeding them up to 50% bunt infected grains over a period of 45 days did not show any adverse effect. Toxicological studies using monkey as an experimental animal also showed that consumption of as high as 70% diseased wheat grain in the diet was not toxic to animals (Bhat *et al*. 1980, 1981). It is apparent from these studies that Karnal bunt does not pose risk to human health and the effect of trimethylamine can be overcome by peeling, debranning and washing of infected wheat lots before grinding. In Mexico, grain lots rejected by milling industry due to greater levels of Karnal bunt infection are used as animal feed (Brennan *et al*. 1990). Nagarajan (2001) observed that infections beyond 2% has consumer rejection as many families in India purchase the grain and milled for use. Since, the teliospores of Karnal bunt do not survive the milling process, the wheat produced from infected area can be converted into flour. The steam flake milling and Holo flite thermal processing used to generate animal feed renders the teliospore non-viable (Bonde *et al*. 1997). This is one viable alternative to avoid the risk associated with Karnal bunt contaminated or infected wheat consignments.

Pathogen

The pathogen was first described as *Tilletia indica* Mitra (1931) but later Mundkur (1944) transferred to genus *Neovossia* and renamed the fungus as *Neovossia indica* (Mitra) Mundkur. Fischer (1953) limiting the species of *Neovossia*, again transferred it to *T. indica*. Krishna and Singh (1982) have shown that *T. indica* is closer to *Neovossia* than to *Tilletia* on the basis of histopathology of sorus development and cytology of germinated teliospores.

The teliospores of *T. indica* are darker than those of *T. caries* and *T. foetida* and are spherical to oval, with reticulations on the epispore and curved spines; measure 22–49 microns (average 35µm) in diameter. The electron microscopic studies on teliospores morphology showed the existence of 3 layers, viz the perisporium, the episporium and the endosporium (Singh 1986). The young spores bear an apiculus arising from the episporium and not from the perisporium. Gardner *et al.* (1983) reported that the sheath of teliospore is fragile and the thick projections arise from the exospore, which appeared to be irregular.

Teliospore germination: The teliospores enable the pathogen to survive during the summer months of May and June when the temperature reaches more than 40°C. Fresh teliospores have dormancy which can be broken by exposing them to high temperature (40–43°C) under direct sunlight for 18 days or more. Soaking teliospores in peptone, wheat straw extract, benzaldehyde, furaldehyde or butyric acid can influence dormancy and only 50% of the teliospores germinate in plain water. If teliospores are buried deep in top soil they retain germinability for 2 years. By keeping fresh teliospores at 15–20°C for 10–15 days and by subjecting the spores to various treatments dormancy gets broken and permitting a free and better germination. After teliospore germination, a stout promycelium is produced with a cluster of 60–185 primary sporidia at the tip. These sensitive, short-lived sporidia germinate in free water and produce a thick mycelical mat. Subsequently, from a cushion-like structure, crescent shaped secondary allantoid sporidia are produced. The secondary sporidia occasionally exhibit yeast-like tendencies to bud and produce another crop of allantoid spores on a wet leaf surface. Depending on temperature and availability of free water, the pathogen follows different pathways to produce crops of spores (Singh 1986, Dhaliwal and Singh 1989 and Smilanick *et al*. 1989).

Pathogenic variability: Variability is a pre-requisite for better survival and co-evolution of organisms. Based on the reaction of various *T. indica* isolates on a set of 18 differentials, four pathotypes (K1, K2, K3 and K4) have been reported (Aujla *et al*. 1987). Since in each annual cycle of the pathogen, nuclear fusion takes place between heterothallic secondary sporidia, hence in *T. indica* the race/pathotype approach is not valid. It is appropriate to classify the variation in isolates as aggressiveness. Following this argument and using a set of overlapping differentials proposed by Aujla *et al.* (1987), five aggressive (Karnal bunt Ag) isolates have been identified by Singh *et al*. (1995) and there is likelihood for the presence of many more. Thirumalaisamy *et al*. (2006) studied the pathogenic variability in *T. indica* isolates collected from north and north-western India on 18 differential hosts and identified three distinct aggressive types Karnal bunt-AgI, Karnal bunt-AgII and Karnal bunt-AgIII from different locations. Aggarwal *et al*. (2010) also further confirmed about three pathotypes existed among the Indian isolates of *T. indica*. Kumar *et al*. (2009) determined eight mating types of *T. indica* based on the artificial inoculation assays using paired monosporidial lines (MLs) raised from haploid allantoids sporidia generated from the teliospores of various places of north-western India and reported about distribution of heterothallic alleles of *T. indica* in India.

Furthermore, isozyme analysis for single spore cultures from Indian and Mexican collections for 48 enzymes have provided fairly good evidence in favour of the existence of genetic variability (Bonde *et al*. 1985). Random amplified polymorphic DNA (RAPD) has been employed successfully to elucidate genetic relationships among different species of *Tilletia*, except *T. indica* (Shi *et al*. 1996). Datta *et al*. (2000) reported a genetic diversity analysis in Indian isolates of *T. indica*. A library of isolate Ni7 was constructed, and three repetitive elements (pnir9, pnir12 and pnir16) were identified for molecular analysis. Immuno-pathotyping of *T. indica* isolates was carried out using anti-mycelial antibodies (Varshney *et al*. 2003). These assay were employed for immune analysis of diversity among Karnal bunt pathogen. Kumar *et al*. (2004) studied the pathogenic and molecular variations in a large number of isolates of *T. indica* by using PAGE and revealed the presence of 3 protein types. Recently, with the help of universal rice primers monosporidial lines of *T. indica* were differentiated and also proved the heterogeneity in the pathogen population (Aggarwal *et al*. 2010).

Epidemiology

It is apparent from disease cycle of Karnal bunt that it is a soil-, seed- and air-borne disease. Teliospores of *T. indica* in the seed get into the soil at the time of harvesting, threshing or winnowing. The spores remain viable for a number of years in the soil lying 15 to 25 cm deep. Teliospores buried deeply in soil losses their viability due to low availability of oxygen and moisture. Under irrigated soil, the spores show significantly higher germination, resulting in higher infection (Gill *et al*. 1993). Irrigation provides the much needed moisture for teliospores to germinate. The chances of survival of teliospores are more under the irrigated conditions of rice (*Oryza sativa* L.)– wheat cropping system than maize (*Zea mays* L.) –wheat cropping system (Sharma *et al.* 2000).

The dormant dark coloured thick walled teliospoes tolerate harsh, warm and dry summer conditions during post-harvest period. The spores germinate from the middle of February to middle of March depending upon suitable meteorological conditions. For teliospores germination optimum temperature range is between 20 and 25°C. It was believed that the teliospores germinate in soil and produce 110–185 primary sickle shaped sporidia which are the infective entities (Singh 1986). But now it has been established that on teliospore germination, two types of sporidia namely allantoid and filiform are produced and they play distinct role (Dhaliwal and Singh 1988) (Fig 2). The filiform sporidia serve as the reproductive bodies to raise allantoid sporidia in successive germinations, whereas the banana shaped allantoid sporidia are infective bodies (Dhaliwal and Singh 1989). Nagarajan (1991) hypothesized that the flag leaf intercepts the allantoid sporidia and facilitate the run down of water droplets with allantoid sporidia into the leaf sheath along with rain. Subsequently, it was shown that the flag leaf sheath acts as the congenial site for allantoid sporidia multiplication and inoculum build-up. The awn emergence stage corresponding to Z49 is the most vulnerable stage for the rain promoted inoculum run down (Kumar and Nagarajan 1998).

The establishment of the pathogen is highly dependent on suitable weather conditions. The infection gets established very well if the maximum temperature is in the range of 19–23°C and minimum 8–10°C, followed by high humidity by intermittent rains. These meteorological conditions prevail in the north-western region to cause more infection but absent in central and peninsular India (Gill *et al.* 1993, Singh and Srivastava 2001). Workneh *et al.* (2008) determined the effect

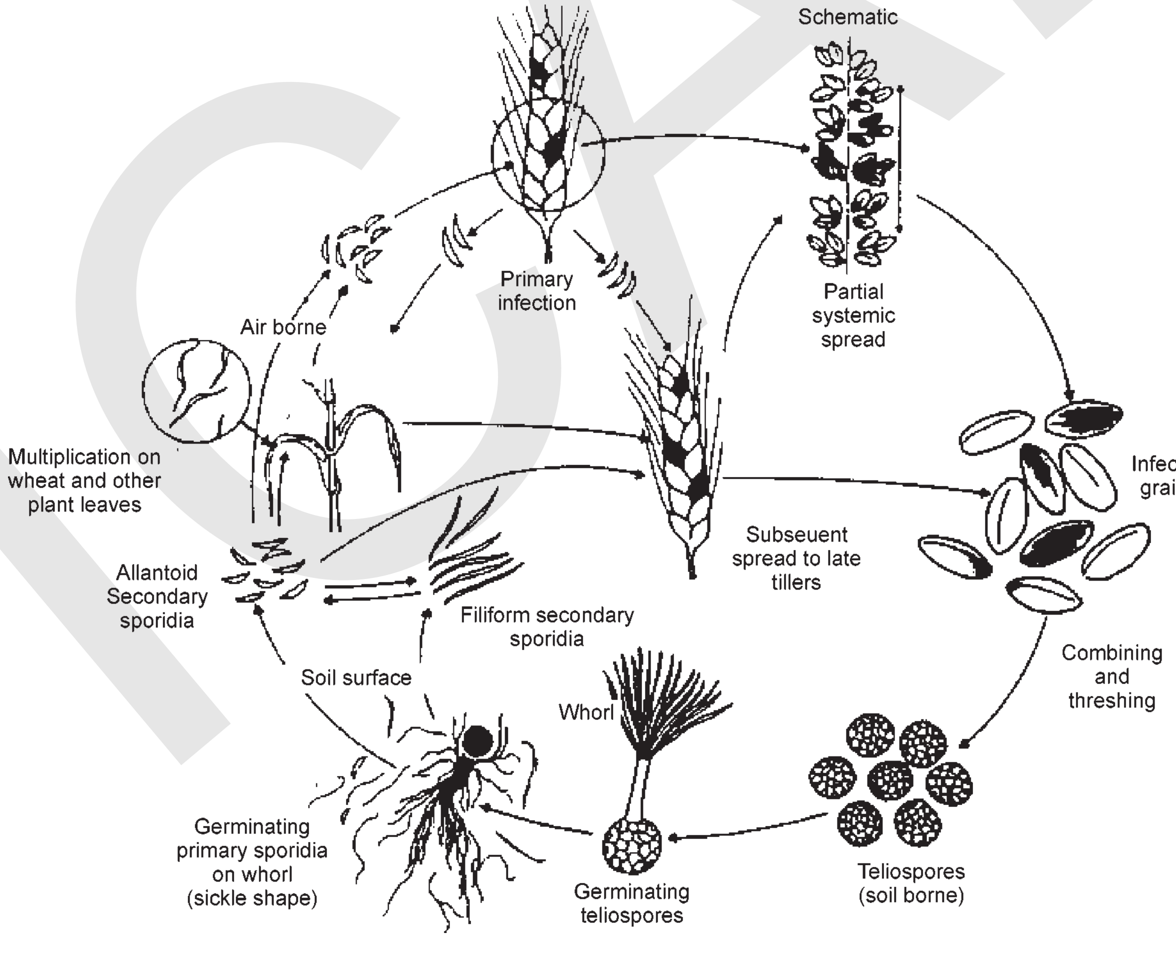

Fig 3 Life-cycle of *Tilletia indica* (Dhaliwal and Singh 1988)

of weather factors on Karnal bunt in Texas (USA) and found three weather variables namely temperature, amount and frequency of rainfall correctly predict the occurrence of the disease in field. Karnal bunt cannot establish and survive in the mid and higher altitudes of Himalayas, where snowfall occurs every year. In this region, the snowing and thawing reduce the viability of teliospores in soil and possibly snowing also induces cold dormancy and therefore, the chilled spores require more energy to germinate. The spells of snow can delay the process of teliospore germination, sporidial proliferation and ultimately result in disease escape (Siddhartha *et al*. 1995).

Bonde *et al.* (2004) studied the survival of teliospores of *T. indica* in states of Kansas, Maryland, Georgia and Arizona of USA in different soils at different depths and hypothesized that soils irrespective of weather, affect teliospore longevity. Badadoost *et al.* (2004) also reported that teliospores of *T. indica* can survive in soil of Montana, USA for more than 32 months. There is a great impact of soil composition on teliospore survival and also it was found significantly, positively correlated to the number of times a field had tested Karnal bunt positive (Stein *et al.* 2005). Ali and Farrokhi (2007) reported that the teliosporogenous mycelia of *T. indica* survive in infected wheat grains for several months in storage.

Since soil-borne primary inoculum of Karnal bunt is the source for the annual recurrence of the disease, a procedure was developed to quantify the teliospores population in soil by bubbling floatation technique using mineral oil with 25% glycerol and developed a linear equation to predict soil-borne inoculum (Singh *et al*. 1990). The severe infection at Gurdaspur and Dhaulakuon (Himachal Pradesh) was attributed to the presence of high soil inoculum as compared to Saharanpur, Hisar and Dehradun, where both teliospore inoculum and disease were less (Singh *et al.* 1996).

Management strategies

Karnal bunt perpetuate year after year through seed-, soil- and air-borne inoculum. Sporidia formed on the soil surface after germination of teliospores become air-borne by wind currents or water splash and infect the developing grains in the ear head. In nature, these sporidia produce superficial colonies on the host surface and generate more sporida to cause floral infection (Dhaliwal and Singh 1988). Such interaction of the life-cycle of *T. indica* suggests that the integrated management approach is most ideal for reducing the disease. Some of the effective cultural, chemical and biological approaches have been enlisted in Table 1.

Disease prediction is of fundamental importance for successful and economical use of fungitoxicants against any disease. Singh *et al*. (1990) attempted to develop a linear model for prediction of the frequency and severity of Karnal bunt. From the linear model it appears that maximum and minimum temperatures and rainfall play a vital role in the development of the disease. If these weather conditions are thoroughly monitored, one can roughly predict the Karnal bunt incidence and severity for a given or localized area. Mavi *et al*.(1992) have utilized the forecasting models for effective application of fungitoxicants in the management of the disease. An attempt was also made by Nagarajan (1991) to predict Karnal bunt based on climatic and field data from India and Mexico. The Mexican prediction system was validated for 1990 and the results agreed with the prediction.

Genetical approach: Use of resistant varieties is the most economical and powerful approach to manage Karnal bunt. Unfortunately, immunity to Karnal bunt in *T. aestivum* varieties is lacking. During the 1970s many of the widely cultivated semi dwarf wheat varieties such as 'HD 2009', 'WL 711', 'UP 262' etc. were highly susceptible as they were not selected for Karnal bunt resistance. But when Karnal bunt becomes endemic over north-western India, varietal screening was intensified and some tolerant bread wheat genotypes 'WL 1562', 'PBW 154' and 'HD 2281' including durums 'PBW 34', 'DWL 5023', 'PBW 215' and 'PDW 233' were released. A large collection of wheat germplasm was field inoculated and evaluated at many centres for resistance based on the number of spikes infected and the type of sorus produced and as a result a number of bread wheat were identified as resistant, namely 'HD 29', 'HD 2300', 'HD 2499', 'WL 7104', 'Raj 2071', 'HW 1045', 'HS 346', 'HP 1531', 'PBW 1154', 'HUW 453', 'HD 4564', 'HD 4571', 'MG 173-1-2-4', 'DWL 5010', 'DWL 1023', 'PBW 215', 'PBW 233', 'Raj 1707', 'Raj 2296', and 'WH 805'. (Gill *et al*. 1993, Singh *et al.* 1998). Sharma *et al.* (2001) developed six Karnal bunt-free lines ('KBRL 10', 'KBRL 13', 'KBRL 15', 'KBRL 18', 'KBRL 22', and 'KBRL 24') and three high-yielding Karnal bunt-resistant wheat ('W 7952', 'W 8086' and 'W 8618') by pyramiding resistance genes and pedigree method of breeding, respectively. A large number of wheat lines tested in Mexico, most were susceptible, but several lines in all market classes had good resistance (Singh and Rajaram 2006).

Multiple resistance to Karnal bunt and rusts was recorded in wheat strain 'DL 377-8', 'DL 802-3', 'DL 790-1', 'DL 330-1', 'DL 484-2', 'DL 790-2', 'DL 770-1', and 'DL 760-1'. Also some wheat species and amphidiploids [*Triticum tauschii*, *T. dicoccoides*, *T. aestivum* cv. Chinese spring, *T. spelta album*, *T. spelta* grey, Amphidiploides : Chinese spring/ *Agropyron elongatum* (2n = 56), Chinese spring /*A. junceum* (2n = 56)] may provide an effective source of resistance (Singh *et al*. 1998).

Genetics of resistance: The knowledge of genetic resistance is essential for breeding disease-resistant varieties. Genetic analysis shows that 'HD 2329', when used as the recurrent parent in a back cross programme, gives more Karnal bunt resistant progenies. Monosomic analysis using three resistant lines 'HD 29', 'WL 6975' and 'WL 2348' and the susceptible 'WL 711' shows that the gene

Table 1 Cultural, chemical and biological approaches to tackle Karnal bunt disease of wheat

Management approaches	Effect	References
Cultural		
Crop rotation	Lowering the disease incidence	Singh 1986
Non-cultivation of wheat for two consecutive years	Effect on primary soil-borne inoculum	Singh 1986
Adjustment of water and fertilizer balance	Lowering the disease incidence	Gill *et al.* 1993
Adjustment of sowing dates	Influence the incidence	Gill *et al.* 1993
Soil solarization for 21–28 days during summer using plastic sheet	Teliospores become non-viable due to high soil temperature	Gill *et al.* 1993, Singh *et al.* 1998
Growing of chickpea in between wheat rows	Minimize soil-borne inoculum	Singh *et al.* 1998
Soil amendment with decomposable organic matters, sugarcane refuge, different cakes, saw dust and wheat straw	Minimize soil-borne inoculum and inhibition of teliospore germination	Singh 1994, Singh *et al.* 1998
Cropping system maize–wheat is better than rice wheat	Less teliosporic counts in soil	Singh *et al.* 1998
Tillage systems: Zero tillage in rice–wheat cropping system	Lowest incidence of disease	Sharma *et al.* 2007
Tillage of highly infested soil	Effects dispersion of teliospores	Allen *et al.* 2008
Chemical		
1. *Seed treatment:* Dry or slurry seed dressing with aureofungin, ethyl mercury chloride, ethyl mercury phosphate, fentin acetate, triphenyltin chloride, fentin hydroxide, oxycarboxin, benomyl, carbendazim, triadimenol, methfuroxan, tetramethylthiuram disulphide, carboxin and indar	Fungistatic action on teliospores contaminating the seeds. Complete killing of the teliospores not achieved	Gill *et al.* 1993
2. *Foliar sprays:* a. Mancozeb (0.25%), Carbendazim (0.1%), Triadimefon (0.2%) Propiconazole (Tilt), cyproconazole (SAN 619 F) and diniconazole (S 3308) at heading stage. b. Folicur (0.2%), contaf (0.1%), tilt (0.1%) and 100g a.i. thifluzamide/ha. c. Weedicides: Application of isoproturon, stomp and 2,4-D	Prevent floral infection of wheat. In case of new fungicides, viz diniconazole, propiconazole, folicur, contaf etc. there was no detectable residues of in wheat straw at harvest. Folicur, contaf, tilt and thifluzamide provide more than 90% control Weedicides inhibit germination of teliospores and sporidia. Stomp is inhibitory even after 96 days to escape infection at boot leaf stage	a. Gill *et al.* 1993 b. Singh *et al.* 1998 c. Singh *et al.* 1998
Biological		
Antagonistic fungi *Trichoderma viride*, *T. harzianum*, and *Gliocladium delinquescens*	Inhibit germination of teliospores of *T. indica*	Singh *et al.* 1998
Trichoderma pseudokoningii, *T. lignorum*, *T. koningii*, *G. deliquescens* and *G. virens*	Antagonize teliospores and secondary sporidia and inhibit their germination *T. lignorum* stimulate the growth of wheat plants	Amer *et al*. 1998
Phytoextracts: Dichloromethane (DCM) extract of *Chenopodium ambrosiodes*, *Encelia farinosa* and *Larrea tridentate* (500 mg/ml).	Reduced radial mycelial growth and found total inhibition.Markedly differed in their fungitoxicity to *T. indica*	Rivera Castaneda *et al*. 2001
Leaf extract of *Vitex negundo*, *Cassia fistula*, *Azadirachta indica*, *Eucalyptus tereticornes* and *Lantana camara*		Sharma and Basandrai 2004

governing resistance to Karnal bunt is located on chromosomes 1D, 2D, 3B, 3D, 5B and 7A (Sawhney and Sharma 1996).

Singh *et al*. (1995) reported that digenic genotypes such as 'Luan', 'Attila', 'Vee 7/Bow', 'Star', 'Weaver', 'Milan', 'Turacio', 'Opata', 'Picus' and 'Yaco' had a higher level of resistance to Karnal bunt than those lines with a single gene. According to Fuentes-Davila (1998), Karnal bunt resistance appeared to be based on few partially dominant or partially recessive genes and is additive. It was also reported additive genes at nine loci governing Karnal bunt resistance in common wheat cultivars. Using 2 RIL (Recombinant Inbred Lines) populations derived from crosses of two resistant donors, 'HD 29' and 'W 485' with susceptible cultivar 'WH 542', it was further confirmed that Karnal bunt resistance is generally based on two or more genes with additive effects (Sirari *et al.* 2008). Dhillon *et al*. (2006) conducted genetic analysis in bread wheat population developed for incorporating Karnal bunt and leaf rust resistance in 'PBW 343'. The F2 segregation revealed two additive genes for

Karnal bunt and complete resistance to leaf rust on account of *Lr24*.

Bag *et al.* (1999) have shown that resistance to Karnal bunt in 'HD 29', 'HP 1531', 'W 485' is conditioned by a single recessive gene and all the three accessions carry a non-allelic gene for resistance. The gene for resistance was located on chromosome 2D of 'HD 29'. Based on the crosses of same parents, Swati and Goel (2010) reported that Karnal bunt resistance is controlled by two dominant genes and the genes interacted with each other in dominant recessive manner. In a PCR-RAPD study, Gogoi *et al.* (2005) found a PCR product of 1.0 kb from resistant cultivars 'HD 29' and 'DWL 5023' and possibility of its linkage with Karnal bunt resistance gene. Purwar *et al.* (2010) clearly observed the role of cystatin gene family (*WC1*, *WC2* and *WC4*) in differential and stage dependent immunity against Karnal bunt disease. They showed that *WC1* protein was abundantly expressed in resistant genotype and high expression was observed at the S2 stage as compared to susceptible genotype. Based on the presence of markers like cystatins which are involved in defence, identification of resistant wheat germplasm can prove to be an important strategy for successful Karnal bunt management (Gupta *et al.* 2010).

Mujeeb-Kazi *et al*. (2006) studied Karnal bunt resistance in synthetic hexaploid wheat (SH) derived from durum wheat × *Aegilops tauschii* combinations and in some SH × bread wheat derivatives. They concluded that resistance exhibited by SH wheat has been transferred into elite but Karnal bunt susceptible bread wheat cultivars thus generating a new unique genetic resource that can be readily exploited by conventional breeding programme.

Singh *et al.* (2003) could map resistance gene in RIL of wheat using 90 SSRs and 81 AFLP markers. In addition, they reported a micro-satellite marker, *Xgwm538*, associated with a (QTL) for Karnal bunt resistance. This marker was further improved by Brooks *et al.* (2006) into a single nucleotide polymorphism (SNP)-based marker. Singh *et al.* (2006) identified Karnal bunt resistance QTL in two recombinant inbred mapping populations, viz population-1 ('WH 542'/ 'HD 29') on the chromosome 4B, 5B and 6B and population-2 ('WH 542'/ 'W 485') on the chromosome 4B and 6A. Sehgal *et al*. (2008) developed Karnal bunt-resistant near isogenic lines (NILs) and utilized 400 SSR markers for their screening. They found presence of donor alleles of four markers: *Xgwm99*(1AL), *Xgwm149*(4BL), *Xgwm174*(5DL) and *Xgwm340*(3BL) in the resistant pool. Kumar *et al*. (2007) developed 104 wheat RILs developed from a cross between parents resistant ('HD 29') and susceptible ('WH 542') to *T. indica* and used for identify SSR markers linked with Karnal bunt resistance. Two molecular markers, *Xgwm 37–1D* and *Xgwm 637–4A* showed apparent linkage with resistance to Karnal bunt.

Seed certification

For limiting the entry of the pathogen to disease-free areas within India through either infected or contaminated seeds, stringent seed health standards have been established. The seed certification programme lays down maximum of 0.05 and 0.25% level of Karnal bunt infection for foundation and certified seeds, respectively (Tunwar and Singh 1988). The seed washing test is the most efficient method to quantify the externally seed-borne teliospores. If teliospores contamination is above 25 spores/grain, the seed lot is rated as contaminated (Agarwal and Verma 1979).

Quarantine

The pathogen *T. indica* was intercepted on wheat seed entering the USA from India and Afghanistan (Loke and Watson 1975). Similarly, in India *T. indica* was intercepted on wheat seed from Lebanon, Sweden, Syria and Turkey (Nath *et al*. 1981), but establishment in these countries has not been reported. To avoid the establishment of the pathogen in the United States, Canada, Russia and China, strict quarantine regulations have been imposed on the import of bunted wheat (Singh *et al.* 1998).

To check the spread of the disease within Mexico the Government imposed legal restrictions during 1983/84 on the cultivation of bread wheat in the areas where disease was found prevalent. The whole of the Yaqui valley was divided into 2 km^2. blocks and the farmers could plant wheat in the blocks where disease was less than 1% in the previous year. Only duram wheat was permitted in the blocks with 1–2% infection and only triticale was allowed to be grown where the infection exceeded 2%. By 1987, about 3% of bread wheat area was replaced by duram wheat in the Yaqui valley (Lira 1984).

Detection and diagnostics

A simple technique for the detection of Karnal bunt infection in wheat seed sample was developed by soaking the sample in 0.2% NaOH solution for 24 hr at 20°C (Agarwal and Verma 1983). Dowell *et al*. (2002) developed high speed optical sorting method to detect wheat grains infected with Karnal bunt for screening a large number of bulk samples. This technology provided wheat industry with a tool to rapidly inspect samples to aid in regulating Karnal bunt , and to remove bunted grains from those wheat used for seed, food and feed. Thinggaard and Leth (2003) developed a staining method using the fluorochrome acridine orange (AO) for determination of the viability of teliospores in soil. It was used in the quarantine inspection of seed lots for routine testing of viability of teliospors of *T. indica*. Chesmore *et al*. (2003) developed an image analysis system for the use on bleached teliospores of *T. indica* and accuracy of 97% was obtained in separating *T. indica* and *T. walkeri.*

Kumar *et al*. (1998) developed a seed immunoblot assay for detection of Karnal bunt using polyc1onal antibodies. Kutilek *et al.* (2001) conducted a serological-based technique using the protein profiling of *T. indica* by SDS-PAGE and compared the protein profiles of *T. barclayana, T. tritici, T. controversa* and *T. laevis,* but could not differentiate the *T. indica* from other species. Kesari *et al*. (2004) considered the dyed latex bead agglutination test for immunodiagnosis of Karnal bunt to be better for detection or solubilised teliosporic antigens over intact teliospores of Karnal bunt.

Mishra *et al*. (2002) developed RAPD-PCR based molecular technique for identification and discrimination of quarantined and non-quarantined *Tilletia* species using arbitrary primers and differentiated *T. indica* from *T. barclayana*. However, random primers are not reliable for accurate and unambiguous species differentiation. Levy *et al.* (2001) used PCR-RFLP techniques to differentiate *T. walkeri* and *T. indica* based on amplification of Internal Transcribed Spacer (ITS) region with universal primers ITS 5 and ITS 4 and restriction with *Sac1* enzyme but could not differentiate these two species.

A protocol for isolation of species-specific mtDNA from *T. indica* was developed (Ferreira *et al.* 1996). Two primers that specifically hybridize to *T. indica* DNA sequences were developed from *Dra 1* restricted mitochondrial DNA and used for its detection (Smith *et al.* 1996). Frederick *et al.* (2000) reported that the primers developed by Ferreira *et al.* (1996) could not differentiate *T. indica* and rye grass bunt, *T. walkeri*. The primer sets developed from mtDNA amplified germinated teliospores, but could not amplify the ungerminated teliospores. They further developed another five sets of PCR- primers specific to *T. indica* and three sets specifically for *T. walkeri* based on single nucleotide difference. However, amplification of single ungerminated teliospore with the specific primers was not achieved, which is essential for Karnal bunt-free wheat trade.

Accurate and unambiguous detection of single *T. indica* teliospores on wheat seeds as contaminants is still challengeable. Hence, for the detection of Karnal bunt teliospores on wheat seeds, Polymerase Chain Reaction (PCR)-based method with species-specific primers was developed from rDNA-ITS region. Two primers, viz forward ITS K B1 and reverse ITS KB 2 primers could amplify 570bp amplicon uniformly all the isolates of *T. indica* alone, but not the other species like *T. horrida* and *T. caries*. He further used these specific primer and could amplify DNA isolated from 500 teliospores of *T. indica* from seed lots. This is the first report of species-specific primers developed from the rDNA-ITS region. The sensitivity of ITS primers (ITS KB 1 and ITS KB 2) was further established by detecting the specific amplification of 570bp from a minimum of 10 teliospores only (Thirumalaisamy *et al*. 2008). Tan and Murray (2006) also developed a two-step PCR protocol using fluorescence resonance energy transfer (FRET) probes for direct detection and identification of *T. indica* from a very few number (□10) of spores.

Pest risk analysis

In the wake of WTO agreement, Pest Risk Analysis (PRA) is essential for prediction as well as management of any quarantined pathogen or pest. PRA has three interrelated elements namely initiation, risk assessment and risk management, one following to other in assessing risk involved in moving wheat from one place of surplus to the zone where it is important for usage (FAO 1996). Several prediction models have been developed for India (Singh *et al.* 1990, Nagarajan 1991, Mavi *et al.* 1992). All these models are based on single location multi-year information. Nagarajan (2001) developed PRA models, GEOKB and KBRISK to predict Karnal bunt risk. Several other countries have also developed PRA models for Karnal bunt, Monte-Carlo model was developed for USA (Podleckis and Michael 1998), Best-fit model for UK and European Union (Sansford 1998). Australia, which is a Karnal bunt-free continent, has developed prediction and risk assessment model to protect wheat from the Karnal bunt (Stansbury and McKindy 2002).

Baker *et al.* (2005) tried to combine disease model with crop phenology model to assess the risk to *T. indica* in European countries. The result indicated that arable areas of western and central Europe, particularly France was found to be best suitable for getting infection on bread and feed wheat, while the northern Italian plains for wheat infection. Jones (2009) put arguments based on conditions required for teliospore germination and survival in the soil along with weather parameters and concluded that *T. indica* may not establish in Europe and suggested that pathogen may be reclassified as cause of minor disease that is likely to have little quarantine significance for Europe and North America. But Inman *et al*. (2008) studied the survival of teliospore at three European sites (Norway, U.K. and Italy) for three consecutive years and indicated that teliospores of *T. indica* can survive for at least 3 years in European soils. This prolonged period of survival could support establishment of the pathogen if is introduced to European wheat production areas. But on the basis of the impact of climate change, Dumalasova and Bartos (2009) contradicted as in Europe the precipitations are distributed more uniformly, they are less evaporated, moisture is available for longer time. These conditions support microbial antagonism and teliospores degradation. Also cooler conditions and more frequent rainfalls probably trigger 'suicidal' germination of teliospores at the time not suitable for infection.

Future thrust

There are some gray areas which need to be addressed to understand the complex life-cycle of the Karnal bunt pathogen with ultimate aim to manage the disease in an effective manner: (i) estimation of threshold level of soil-borne inoculum that causes primary infection in the wheat crop, (ii) viability of secondary sporidia on soil, host and air in relation to micro and macro-climatic conditions, (iii) to exploit biochemical and morphological components for developing resistant stocks, (iv) a wide range of pathogenic and molecular variability has been reported in *T. indica* and there is need to generate uniform information for durable resistance, (v) pyramiding effective resistance genes from various sources against Karnal bunt in desirable genotypes, (vi) bioclimatological models based on the relationship between weather parameters and disease dynamics should be validated, and (vii) as Karnal bunt is being increasingly used as an instrument for non-tariff barrier, a reliable molecular diagnostic technique should be developed for defection of *T. indica* for issuing sanitary and phytosanitary certificate.

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