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Karnal bunt resistance in wheat – an overview

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ABSTRACT

Although the disease was first identified as early as 1931, the sporadic work carried out upto 1980 lacked continuity in research to prove or disprove the observations in relation to resistance. Wide adoption of syringe inoculation method devised at PAU, Ludhiana, lead to intensive and extensive screening of germplasm at five centres (Pantnagar, Dhaulakuan, Delhi, Hisar and Karnal) in India under AICW&BIP and at CIMMYT. This lead to identification of several KB resistant stocks. Use of progeny based KB data allowed qualitative genetic analysis of resistance. This was followed up by RIL based studies which allowed multiple screenings and more precise genetic analysis. Most of the studies revealed that 2-3 additive loci govern resistance in the resistant stocks, with resistance alleles being partially dominant. Further the resistant stocks have been shown to carry diverse genes for resistance. As many as nine different loci were identified in a set of four resistant stocks. High degree of resistance is required in context with quarantine and quality implication of KB. Initially synthetic hexaploids developed at CIMMYT were shown to possess high degree of resistance. KB-free lines have been developed in bread wheat in India by crossing resistant stocks. Concerted efforts are underway to incorporate resistance from KB-free lines and diverse sources (e.g. ALDAN'S'/IAS 58, CMH 77.308, H 567.71/3*PAR, HD 29, HP 1531 and W 485) into commercial and advanced lines through limited backcross approach. Presently material developed with this approach is being evaluated in multilocation yield trials. Using marker approach in synthetic wheats resistance has been shown on 3 BS and 5AL whereas in bread wheat lines, HD 29 and W 485 on 4 B, 5B and 6B. Studies are in progress to identify molecular markers in near isogenic lines developed from the cross KBRL 22 (a KB-free line) and PBW 343. The MAS worthy markers, however, remain to be developed as QTL of large effects need to be identified and tightly linked with DNA tags. To simplify resistance gene tagging and pave way for subsequent molecular characterization the NIL approach is being adopted e.g. NIL and micro RIL populations have been developed from cross of KBRL 22 (a KB-free line and PBW 343)

Key Word: Wheat, Karnal bunt, *Tilletia indica*

Karnal bunt (KB) of wheat caused by *Tilletia indica* has a great significance in global wheat trade because of stringent quarantine measures as the disease spread through infested/infected seeds. The KB infected grains emit fishy odour due to the presence of trimethyl amine and produce black powdery mass of teliospores impairing the quality of wheat and its products. Around 16-19 % of the world wheat produce is traded annually between the countries. Inability to ensure KB free produce can foil exports from India, the second largest producer of wheat in the world.

The disease was first identified in 1931 in wheat fields of Karnal, India (Mitra 1931). Howard and Howard described a similar bunt disease from Lyallpur (Faisalabad) Pakistan in 1909 but did not ascertain the pathogen identity (Gill *et al.* 1993). Later, during 1930s and 40s it was also reported from Punjab and South Western parts of Pakistan. In different years the disease was reported from several other parts of the world which includes, Afghanistan, and Iraq (Locke and Watson 1975, Williams 1983, Warham 1986, Gill *et al.* 1993), Mexico (Duran and Cromarty 1977), Iran (Torabi *et al.* 1996), Nepal (Singh *et al.* 1989), United States (Ykema *et al.* 1996, Perring *et al.* 1996, Dowell *et al.* 2002) and South Africa (Crous *et al.* 2000, Boucher 2002). The disease is not detected from Sweden, Turkey and Lebanon, though the pathogen was intercepted in the seeds imported by these countries.

Mature teliospore of *T. indica*, the oversummering propagules of the pathogen contains a single diploid nucleus, which on germination divides meiotically, produce basidiospores that form hyphae, allantoid sporidia and successive generations of sporidia which undergo dikaryotization at some stage of the life cycle to produce diploid telospores. Infection occurs when sporidia land on spikes, germinate to produce hyphae which penetrate either through nucellus or directly through the pericarp of young ovaries or stomata of the glumes. The disease finally manifests through formation of teliospores in the middle layers of the pericarp.

In the last 25 years, KB research got great impetus and elaborated studies have been carried out on all aspects of the disease including epidemiology, pathogen biology, host-pathogen interaction (at histopathological/genetic/molecular level). Experiments have been carried out on basic and applied aspects of disease management (by cultural practices, use of chemicals, biological antagonists, plant extracts, unconventional methods-irradiation/ ultrasonic vibrations). However host-resistance has received a greater emphasis. Recently Carris *et al.* (2006) reviewed discussed history, systematics and biology highlighting controversial generic placements and evolutionary linkages between *T. indica* and *T. horrida* in light of knowledge at molecular level.

Since 1980, studies on resistance were taken up extensively in India and at International Centre for Maize and Wheat Improvement (CIMMYT), Mexico. Last decade has seen several publications on diversity analysis, resistance introgression into promising high yielding

genotypes and tagging of KB resistance genes. Studies were also initiated on whether pathogenic variability gets affected after each life cycle because of heterothallic nature of the fungus and what problems can it pose in resistance breeding, if there are multiple alleles for compatibility within and between populations. All these aspects are being reviewed in this article.

STATUS OF KB IN INDIA

Overall, in India the disease intensity is relatively low in North Eastern and Central Zones whereas Peninsular Zone and higher hilly areas of North and South continue to remain KB free (Fig.1). It is, however, widely prevalent in North Western Plains Zone (NWPZ), the major wheat producing region of the country (Sharma 2001).

Ever since the first report of KB occurrence by Mitra (1931), the disease has been appearing at regular intervals. High disease incidence was recorded in 1930s and 1940s when indigenous tall varieties were grown (Gill *et al.* 1993). With the introduction of dwarf varieties during late 1960s an era of intensive and extensive cultivation of wheat was set in and entire wheat belt was occupied by number of varieties viz;

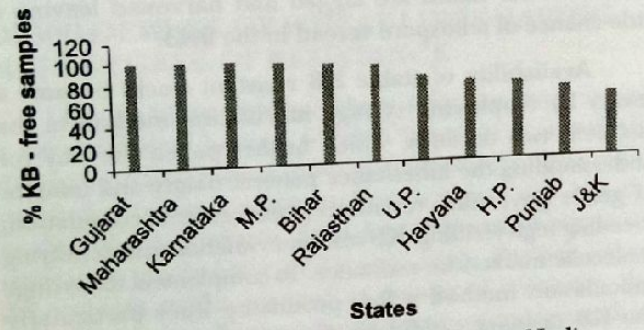

Fig. 1. Karnal bunt free samples in States of India (1994-2007)

Kalayansona, Sonalika, PV 18, WG 357, WG 377 etc. During this period the disease was recorded in high proportions in some of the seasons. Later, The disease incidence further continued to remain high during 1980s in the wake of cultivation of susceptible varieties, WL 711, HD 2009 and WH 147. The introduction and extensive cultivation of variety, HD 2329 in late 1980s resulted in low disease intensity (% grain infection) but without any difference in the prevalence. From 1990 to 2004, the disease was low to moderate in Punjab (Fig. 2), except for 1996 when it was in high intensity (Sharma

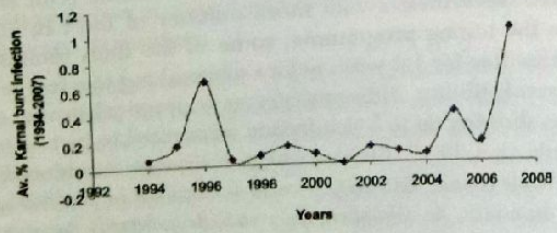

Fig. 2: Average Karnal bunt infection in Punjab from 1994-2007

et al. 2004). This decline was largely concomitant with the large scale adoption of PBW 343 which had relatively low disease score. However, since 2005, a successive increase in the disease levels has been registered with very high incidence during 2007, probably due to preferential multiplication of those components of pathogen population which are better adapted to PBW 343.

As far as India's participation in the global wheat trade is concerned the disease free wheat can be procured from the States of Gujarat, Maharashtra, M. P. and Bihar which are free from KB (Fig 1). Intensive surveys have even led to identification of low incidence pockets within the wider KB affected zones. For instance from Punjab, very less disease has been recorded from the districts of Amritsar, Fatehgarh Sahib and Patiala (Fig. 3). Contract farming (entailing preventive management practices at farm level) and segregating wheat at grain market level may serve as other strategies to ensure KB-free produce for export. Application of chemicals/ultrasonic vibrations/ specific infra-red frequencies for eradicating either seed or soil or air-borne inoculum are highly cumbersome methods and may not find much application and wide acceptability with farmers. Resistance breeding is the ultimate long term strategy to combat the disease.

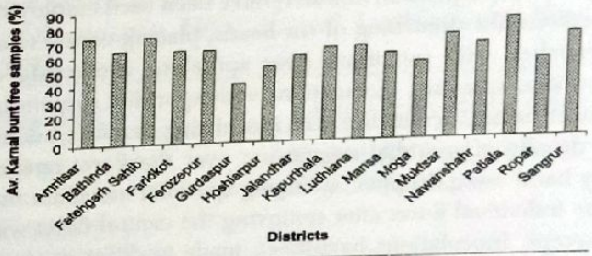

Fig. 3. Karnal bunt free samples in districts of Punjab (1994-2007)

SCREENING FOR KB RESISTANCE

Genetic resistance is regarded as the main option for management of KB as the teliospores of the pathogen survive for 3-7 years under different field and storage conditions. The infection occurs by the air borne allantoid shaped sporidia. There is no chemical available to eradicate the seed as well as soil borne inoculum and the fumigants effective against the soil borne teliospores can not be applied in large areas. Triazole group of chemicals are effective in reducing the disease but their application in the field is cumbersome. Devising screening methodology is a critical step towards the development of disease resistant varieties.

The work on varietal screening was initiated as early as in 1949 at Gurdaspur, Punjab where *T. durum* was found to be free from KB, whereas, cultivar, C 253 recorded 9 % infection (Bedi *et al.* 1949). Later, Chona *et al.* (1961) and Munjal (1971) also indicated resistance in some of the lines screened under normal field conditions as well as by employing various artificial techniques under controlled conditions. In 1979-80 KB screening of Advanced trial

material was initiated under All India Coordinated Wheat Improvement Project (AICWIP), however, no perceptible head way could be made in the absence of artificial inoculation method.

During 1980-81, syringe inoculation method was successfully employed at Punjab Agricultural University (PAU), Ludhiana to screen wheat lines under field conditions. Sporidial cultures were prepared by dusting dry teliospores from infected grains on to Potato - Dextrose - Agar (PDA) medium and also in broth. The sporidial cultures were maintained by frequent subculturing in PDA. Sporidial suspension having >10, 000 sporidia/ml was used for inoculations by putting 1-2 ml suspension at boot stage when ear head is completely enclosed in boot leaf sheath and emerging awns are visible along the flag leaf (Aujla *et al.* 1980, 1982). Adequate humidity to the inoculated plants was provided using mist sprayer in the field. The technique was soon taken up widely in resistance breeding programmes in India and also at CIMMYT. There are number of other reports where screening under natural as well as artificial inoculation conditions was attempted employing various screening methods and different controlled conditions (Aujla *et al.* 1980, 1982, 1983; Singh and Krishna 1982; Krishna and Singh 1983; Warham 1986, 1988, 1990). Pathogen propagules like teliospores, allantoid/filliform sporidia and mycelium (suspension in water) have been used employing methods like drenching of ear-heads, placing cotton swab drenched with inoculum over spikelets, showering of sporidia, spraying, inoculations with sporidial suspension under partial vacuum in a glass inoculating chamber, adding a droplet of sporidial suspension over the floret opened by hand using dropper, syringing sporidial inoculum into the individual floret after removing the central floret with forceps. Inoculations have been made at different stages of ear head growth from early boot to grain formation to identify the disease prone stage. It was, later, realized that use of sprinklers may not be mandatory for syringe inoculations as inoculum suspension is prepared in water (Warham 1986, Pedro *et al.* 2004, Sharma *et al.* 2005).

Concentration of inoculum plays a significant role in determining the level of infection. It is not desirable to use either very high or low densities of sporidia. In a theoretical analysis, Garret and Bowden (2002) have remarked that for high population density, the rate of success of dikaryon formation may be reduced due to limited availability of host tissue whereas for lower densities, availability of potential mates may be reduced.

In winter and spring wheats screening has been done under controlled conditions as a USDA sponsored pre-emptive measure against the disease threat in USA. (Chhuneja *et al.* 2004, Goates 2004). Screening under controlled conditions is, however, cumbersome and may not simulate the natural conditions for differential resistance amongst the lines. In other words, continuous extreme conditions, induced by artificial shading and misting may obliterate the resistance mechanisms. Normally under such conditions high spike sterility is encountered and is likely to bias resistance evaluation. Thus inoculations under normal field conditions are best suited for KB screening provided appropriate stage of inoculation is followed (up to February end/early March). To ensure inoculations during this optimal period in winter and late flowering exotic lines, vernalization and artificial extension of photoperiod may be necessary.

For exact simulation of natural conditions of disease development a method involving addition of teliospores in the field and making them to germinate there and infect the lines to be tested may seem ideal. However, it does not ensure landing of sporidia at vulnerable stage of crop growth i.e. at ear head emergence and thus results in disease escape even in highly susceptible cultivars. This method may also contribute to the disease spread and should not be used widely. Another method of inoculation based on spraying of sporidial inoculum simulates natural conditions of disease development but is difficult to follow on account of the large quantity of inoculum required and a long spraying schedule as the lines show staggered flowering spread over a period from last week of January to March. For Plant breeding oriented work which involves screening a large number of segregating populations, syringe inoculation method has to-date been found to be the most appropriate as all the inoculated ear heads are tagged and harvested leaving a little chance of teliospore spread in the field.

Availability of stable KB resistant stocks became a reality by employing syringe inoculation method in the last over two decades, which further paved the way for understanding the inheritance pattern, nature and number of genes governing resistance, resistance accumulation; breeding high yielding, KB resistant varieties and identifying molecular markers for resistance. To complement the syringe inoculation method a few promising lines particularly the KB resistant candidates for commercial deployment may be evaluated at hot spot locations under natural field conditions.

IDENTIFICATION OF KB RESISTANT WHEAT

In the first year of KB screening under artificial conditions, 286 lines from AICWIP were evaluated at Ludhiana center. Most of the lines scored high disease and only 10 lines showed up to 10% infection (Aujla *et al.* 1980). Artificial inoculations were made using isolates of the pathogen collected from WL711, HD 2009 and WG 357 varieties grown at different agroclimatic zones of Punjab. In the first few years, none of the wheats were found to be highly resistant or immune to the disease (Gill *et al.* 1981). Nevertheless with more number of lines brought into the testing programme, some of the lines remained disease free for 1-3 years before succumbing to a low level of susceptibility. Subsequently as a practical norm, test lines showing up to 5 % infection were rated to be resistant (Aujla *et al.* 1985). Besides wheat, resistance was identified in *Secale cereale*, Triticale, several accessions of *Aegilops spp*, *Ae. biuncialis*, *Ae. columaris*, *Ae. crassa*, *Ae. jubenalis*, Ae. ovata, *Ae. speltoides T. uratu* and *Ae. squarosa* (Warham 1986). As evaluation of advanced breeding material became a regular

programme, KB resistance showed up in several lines and was observed to be highest in Triticale, followed by *T. durum* and *T. aestivum*. The additional D genome in *T. aestivum* was thought to be responsible for KB susceptibility (Aujla *et al.* 1990 a, 1992, Fuentes-Davila and Rajaram 1994, Sharma *et al.* 2000, 2002). Based on screening (at PAU) of more than 45,000 genotypes emanating from the national and international breeding programmes, 835 lines were found to show consistently low disease when screened for 3 to 20 years. These lines were deposited with National bureau of Plant Genetic Resources (NBPGR) for long term storage and utilization along with additional data for rusts and agronomic traits during 2003. One set of these lines has also been put under natural storage at Keylong in Himachal Pradesh. KB resistant lines identified under All India Coordinated Wheat and Barley Improvement Project (AICW & BIP) are included in national genetic stock nursery (NGSN) for utilization by breeders. HD 29 is the most frequently used KB resistant stock, which was first reported from PAU. HD 29 along with another resistant stock HD 30 were registered under AICWIP in the year 1999. Later KB resistant lines of durum wheat (D 482, D 873, D 879, D 895) and a triticale (TL2807) from PAU-Ludhiana were registered with NBPGR.

DEVELOPMENT OF WHEAT LINES HAVING HIGH DEGREE OF RESISTANCE

Unlike other diseases whose infection must exceed a threshold level beyond which it becomes economically damaging, KB incidence above zero falls short of quarantine requirements and a relatively low incidence can ruin the quality of the products. Thus for all practical purposes, resistance levels have to be very high. Further, when KB resistant stocks are used as donors of resistance, the derivatives generally show lesser degree of resistance than the donors -a kind of dilution of resistance in the breeding process is evident. This problem can be resolved to some extent by starting at a very high level of resistance i.e. using highly resistant stocks as donors. High degree of resistance was identified in synthetic hexaploid wheats derived from the crosses of *T. turgidum* and *T. tauschii* (Villareal *et al.* 1996). The durum wheat had low level of infection (0.3 to 0.84 %). Synthetic hexaploids with 0% infection seem to aggregate the resistance from the parental species. Four such synthetic wheat lines, SH12 Altar84/*T.tauschii*-Acc.198), SH46 (Duergand/*T.tauschii*-Acc.221), SH10 (Altar84/*T. tauschii*-Acc.223), SH31 (Chen'S'/*T. tauschii*-Acc.224) were registered. Use of synthetic hexaploid wheats as donors however throws up a large proportion of hard threshing and unadapted derivatives.

Accumulation of diverse genes for resistance represents another option for raising the KB resistance levels in bread wheat. Presence of distinct resistance genes in the donor stocks and prevalence of additive gene action as discussed later under genetics of resistance, makes this a viable option. As a strategy to develop super-resistant stocks at PAU, established KB resistant stocks were crossed and homozygous lines were derived from these resistant x resistant crosses under stringent selection. Selection was aimed at obtaining KB-free plants (having pyramided resistant genes) in contrast to infection levels of upto 5% which could be observed in the parental stocks. Generations were advanced by raising ear to row progenies from disease free plants and evaluating them against mixture and individual isolates of *T. indica* collected from different agroclimatic zones of NWPZ. Several disease free lines were identified and three of these (KBRL 10 derived from HD 29 x HP 1531 and KBRL 13 & KBRL 22 from HD 29 x W 485 cross were registered (Sharma *et al.* 2001, 2002). Thirteen lines from, HD 29 x W 485, were evaluated during 2005 in multilocation trials under All India Coordinated Project and five of them remained disease free. Later, lines showing higher degree of resistance developed from crosses between several other genetically diverse KB resistant stocks (ALDAN 'S'/IAS 58, H 567.71, HP 1531, CMH 77.308) were also tested against the disease under AICW & BIP and 6 of them remained disease free at all the five locations (Table 1). These KB-free lines probably have resistance factor against a wider spectrum of isolates/ environments. One of these lines, KBRL 22 was also used for incorporation of KB resistance in high yielding variety, PBW 343 (Sharma *et al.* 2003).

Table 1 Stocks identified as KB-free on the basis of evaluation under All India Coordinated Wheat and Barley Improvement Project (AICW & BIP) at Dhaulakuan, Hisar, Karnal, Ludhiana and New Delhi

Parental Cross	KB-free lines (designated)
HD 29 x W 485	KBRL8, KBRL14, KBRL16, KBRL22, KBRL24
ALDAN 'S'/IAS 58 x CMH 77.308	KBRL 63
H 567.71/3*PAR x HP 1531	KBRL 68
H 567.71/3*PAR x CPAN 3045	KBRL 67
HD 29 x CPAN 3045	KBRL 69
HD 29 x CMH 77.308	KBRL 70
HD 29 x H 567.71/3*PAR	KBRL 60

GENETIC BASIS OF KB RESISTANCE

Initial work on genetic analysis was based on quantitative genetic methods as distinct classes for resistance and susceptibility were hard to make out. In an early study (Chand *et al.* 1989), both additive and dominance gene effects were identified by the analysis of F1, F2 and F3 generations from a diallel cross involving 10 parents (WL 711 and HD 2009-KB susceptible; WL 2217, UP 1008, WL 1562, SONALIKA, VL 421, HB 208, TzPP, WG 2038 –KB resistant).

Resistance was shown to be dominant in the four intervarietal crosses involving KB resistant (HD 29 and WL 6975) and susceptible (WL 711 and HD 2009) parents (Gill *et al.* 1990). The additive component was significant. Sharma *et al.* (1991) had shown partially dominant to dominant nature of resistance in a set of 36 F1s from a diallel crosses of 9

wheat lines including four resistant (Fec 28, Cebeco 148, HD 29 and HD30), three moderately resistant (DGP 247, WL 6975 and WL 1562) and two susceptible (WL 711 and HD 2009) parents with Gurdaspur isolate. In eight parental (4 KB resistant – HD 29, H 567.71/3*PAR, WL 6856, WL 1786 and 4 susceptible wheats – HD 2329, PBW 344, HD 2009, WH 542) diallel cross analysis. predominant role of additive variation was shown in the inheritance of KB resistance (Nanda *et al.* 1995).

Using qualitative approach, monogenic control of resistance was demonstrated by Morgunov (1994), in all possible crosses of four resistant (WEAVER, W 499, CRUZ ALTA and K 342) and two susceptible parents (LAJ 3302 and WL3399). Weaver, W 499 and CRUZ ALTA were shown to have different genes while CRUZ ALTA and K 342 have same gene for resistance. In the crosses of four synthetic hexaploid wheats and two susceptible wheat cultivars, Seri 82 and Opata 84, F1 hybrids and individual F2 plant derived F3 progenies were screened for KB resistance (Villareal *et al.* 1995). Synthetic hexaploid (SH) wheats were immune to KB whereas susceptible cultivars showed 16.9-17.3 % infection. F1 data suggested the dominant or partially dominant nature of the resistance. The SH cultivars Chen/ *T. tauschii* and Chen/ *T. tauschii* were shown to have a single gene for resistance while 'Altar 84' / *T. tauschii* possessed two. 'Duergand'/ *T. tauschii* //Opata 84 segregated in a ratio of 9:7 showing two complementary dominant genes for resistance.

A series of inheritance studies were conducted at CIMMYT involving confirmed sources of resistance in bread wheat. All possible crosses were employed by Singh *et al.* (1995a) in a study of 4 resistant (ROCK// MAYA/ NAC, RC 7201/ 2* BR2, ALDAN'S'/IAS 58 and Shanghai # 7) and one susceptible parent, WL 711. Proportion of homozygous susceptible progenies in F3 of each cross was used for estimating the number of loci contributing to resistance. In RC 7201/ 2* BR2 and ROCK// MAYA/ NAC, the proportion of homozygous susceptible lines were in accordance with those expected for segregation at one locus. In case of Shanghai # 7, two loci for resistance were implicated while ALDAN'S' / IAS 58 possessed KB resistance genes at three independent loci. In resistant x resistant crosses, no susceptible F3 line indicated at least one common gene present in all the four resistant parents. In another study (Singh *et al.* 1995 b), 14 Mexican genotypes of bread wheat with good to moderate levels of KB resistance were crossed with highly susceptible cultivar, WL 711 to determine the genetics of resistance. The parents, F1, F2 and backcross population were evaluated under artificial epiphytotic conditions during 1993-94 season. KB data of parents and F1 suggested that resistance was dominant to partially dominant. The chi-square analysis of the segregation ratios in the F2 and backcross generations indicated that the resistance in the wheat genotypes; Luan, Attila, Vee # 7/ Bow, Star, Weaver, Milan, Sasia and Turacio/ Chil was controlled by two genes whereas in Cettia, Irena, Turaco, Opata, Picus and Yaco a single dominant gene was responsible. The genotype with two genes for resistance expressed a higher level of resistance than those with a single gene.

The third study in this series was based on seven bread wheat (six resistant and one susceptible) genotypes involving resistant parents originated from China (Shanghai# 8), Brazil (PF 71131),USA (Chris) and Mexico (Amsel, CMH 77.308 and Pigeon) and susceptible line (WL 711) from India (Fuentes-Davila *et al.* 1995). Evaluation of these lines, all possible F1s and large sets of F3s from each cross was carried out for KB resistance. In F3, three classes i.e., homozygous resistant, homozygous susceptible and segregating were made. As all possible crosses were available, inheritance as well as allelic diversity was studied. In this set of lines KB resistance was controlled by partially dominant genes at six loci. The individual stocks had one or two genes for KB resistance. The genes were tentatively designated as Kb 1 in 'Chris', Kb 1 and Kb 2 in 'CMH 77.308' , Kb 3 in 'Amsel', Kb 2 in PF 71131, Kb 2 and Kb 4 in Shanghai # 8, Kb 5 and 6 in Pigeon. Progenies with two genes were relatively more resistant than those with one gene. In this series of studies conducted at CIMMYT classification of progenies into homozygous resistant/susceptible and segregating categories in F3 generation facilitated the manifestation of a qualitative inheritance pattern.

Singh *et al.* (1996) studied the inheritance pattern in the crosses derived from three resistant (HD 29, HP 1531, W 485) and two susceptible line (WL 711 and HD 2329) in F1s, F2s and BC generations, using single isolate, P11. Resistance was dominant, controlled by few major genes, along with minor genes. Some minor genes were present in otherwise susceptible parents.

Perceiving problems associated with environmental influence in KB screening emphasis shifted to use of recombinant inbred lines (RILs) rather than early segregating generations. Harjit Singh *et al.* (1999a) used F8 RILs derived from a resistant (HD 29) and susceptible (WL 711) cross which were inoculated with T. indica isolates Ni 7 and Ni 8. Four genes were observed to be involved in resistance. Three genes conferred resistance to Ni 7 and two genes to Ni 8 whereas one gene has been shown to be effective against both the isolates. The study suggested a gene-for-gene relationship. Bonde *et al.* (1996) showed that KB isolates are not distinct pathotypes but heterogeneous populations. The isolates, however, differ in frequencies of virulence and avirulence alleles at different pathogenecity loci (Datta *et al.* 1999).

In a study by Bag *et al.*(1999) single KB bunt isolate (Delhi) was used to determine the nature and number of genes for resistance in three resistant genotypes of bread wheat (HD 29, HP 1531, and W 485). F1 hybrids between resistant and susceptible parents (WL 711, HD 2009 and HD 2285) showed susceptibility. The F2 population derived from the crosses segregated in a ratio of 3S: 1R and in F3 families of the cross HD 29 X WL 711 and its reciprocal confirmed the findings in F1 and F2 that susceptibility was dominant

over resistance and that resistance was conferred by single recessive gene. It was further confirmed by segregation ratio of 1R:1S in back cross progenies. Results of this study were in disagreement with all other studies and were attributed to the isolate of T. indica deployed for screening.

In a 11 parent diallel analysis which involved six KB resistant (ALDAN, HD 29, W 485, H 567.71, HP 1531, CMH 77.308, two moderately resistant (WL 6975, CPAN 3045) and 3 susceptible (WH 542, UP 2382 and PBW 343) parental lines, additive gene action was observed to be more important in the genetic control of % KB infection whereas dominance gene action was pronounced for coefficient of infection (Sharma *et al.* 2001). Qualitative genetic analysis of these crosses was also carried out and digenic inheritance was indicated in resistant x susceptible crosses. ALDAN possessed some minor genes for susceptibility and WL 6975 & CPAN 3045 expressed differently in different genetic backgrounds and showed variable segregation behaviour from cross to cross (Sharma *et al.* 2004).

An alternative screening system was employed for inheritance study by Kumar *et al.* (2002). Embryos excised from seeds of parents, HD 29 and HD 2329, their F1, F2, BC1, BC2 were cultured on modified MS medium with and without pathogen. An inhibition zone (IZ) was formed around each of the embryo showing callusing. IZ was maximum in HD 29, the KB resistant genotype. Callus response (CR), fresh and dry weight were observed after 30 days of growth. In presence of *T. indica*, additive, and additive x dominance effects for CR, duplicate type of epistasis for fresh weight and dominance x dominance effects for dry weight and additive gene effects for diameter of IZ were observed. However, this system may not be suitable for routine screening work.

In a comprehensive genetic analysis (Sharma *et al.* 2005), genetics of KB resistance has been explored in 10 RIL populations derived from all possible crosses of four well known resistant stocks (HD 29, W 485, ALDAN 'S'/IAS 58, H 567.71/*3 PAR) and a highly susceptible commercial wheat, WH 542. The plant material screened for KB resistance consisted of F2, BC1 and RILs from each of the resistant x susceptible crosses and RILs from the six resistant x resistant crosses besides the parents and F1. The screening was performed under optimal conditions for disease development using a mixture of isolates from NWPZ of India and the widely followed syringe method of inoculations. The F1 of the four resistant x susceptibl' crosses indicated dominance of resistance. Genetic analysis with consistency across the three populations (F2, BC1 and RILs) revealed that parents HD 29, W 485 and ALDAN 'S' / IAS 58 carried two resistance genes whereas three genes were indicated in H 567.71 / *3 PAR. Further, the resistance genes in these parents acted in an additive manner. The six resistant x resistant RILs showed that the genes in the four resistant stocks were different from each other and as many as nine loci govern KB resistance in these stocks. RILS with zero disease score from resistant x resistant crosses gave indications of gene pyramiding effects. This material was later used for molecular gene tagging.

INTERSPECIFIC GENE TRANSFER FOR KB RESISTANCE

Amphiploids were synthesized by crossing *T. durum* with *T. monococcum*, *T. boeoticum* and *Ae. squarrosa* (Gill *et al.* 1988). An amphiploid synthesized by crossing *T. durum x T. monococcum* showed high level of resistance to KB. Resistant F7 lines were derived from crosses of amphiploids with a susceptible genotype, WL 711(Singh *et al.* 2004).

A large number of synthetic hexaploids from *T. turgidum x T. tauschii* were developed and crossed with *T. aestivum* at CIMMYT. These synthetic wheat lines were distributed through international nurseries and are being used for incorporating area specific traits in respective agronomic superior genotypes by limited backcrossing.

Using a RIL population, a saturated molecular genetic map has been developed from *T. monococcum*-Acc. 14087 x *T. boeoticum*-Acc. 5088 which showed at least one gene in each parent for KB resistance (Singh *et al.* 2004).

IMPLICATIONS OF PATHOGEN BIOLOGY IN KB RESISTANCE BREEDING

Aspects of pathogen biology have a direct bearing on making resistance breeding a viable strategy. These issues have not been adequately highlighted in the literature but researchers have responded to these in many ways. The genetic and molecular marker work has almost exclusively shifted to use of host populations such as RILs which allow replicated as well as multiple screening over the years. For instance, we have used three year RIL data for genetic anlysis (Sharma *et al.* 2005) and QTL mapping of KB resistance (Sukhwinder-Singh *et al.* 2007). Further, breeding for KB resistance now lays much less emphasis on early generation selections which would have involved single plant screenings.

On the pathogen side, more homogeneous inoculum systems have been devised. Initially, single isolate-based inoculum system was employed in a few studies which is less heterogeneous than the mixture of several isolates used routinely (Harjit-Singh *et al.* 1999a). Further, single teliospore derived cultures from a single isolate and finally genetically homogenous inoculum system based on single compatible monosporidial pair has been deployed to understand genetics of KB resistance. In a recent study (Table 2), the impact of different levels of genetic homogeneity in the inoculum on the precision of genetic analysis was empirically investigated. RIL populations derived from 3 KB resistant stocks (ALDAN'S'/IAS58, HD 29 and W 485) and one susceptible genotype (WH 542) were evaluated for KB score using: i) a single pair of compatible monosporidia representing a genetically homogenous pathogen population from isolate (P4) ii) a single teliospore of P4 isolate having several pairs with differential pathogenecity- representing heterogeneity within recombinants of a single teliospore iii) mixture of isolates from different agroclimatic regions representing high genetic heterogeneity. In all the RIL populations, 3 loci were identified using genetically homogenous inoculum, whereas 2 were indicated with heterogeneus inoculum. Generally the

use of homogeneous (or single race) inoculum is expected to simplify the genetic analysis. This is typically observed for race specific major gene resistance as in rusts. The contrary results observed in case of KB are due to a completely different mode of resistance gene action compared to the rust resistance major genes. KB resistance is based on more than one gene with additive effects and which are, as indicated here, probably race non-specific. Under these circumstances a mixture of most virulent populations will reveal the smallest number, albeit the most useful of the KB resistance genes. Thus use of homogeneous culture does not allow simplification of genetic analysis or an advantage in screening for plant breeding purposes. Rather inability to represent the pathogen population adequately will pose problems in identification of resistance as it impacts the genetic architecture of pathogen population.

The diploid teliospore of *T. indica* on germination undergoes one meiotic division followed by 5-6 mitotic divisions resulting in about 180 haploid primary sporidia. The secondary sporidia produced serve as the infective agents with the condition that distinct mating types dikaryotize prior to manifestation of infection. Early studies indicated that mating system in *T. indica* is controlled by multiple alleles at one locus (Duran and Cromarty 1977). However, Royer and Rytter (1985) and Aujla and Sharma (1990) suggested the possibility of tetrapolar compatibility system in *T. indica*. Resolving the genetic basis of compatibility system in *T. indica* is important for understanding the dynamics of pathogen variability which has a bearing on the screening system for resistance breeding. Another important issue that remains unclear is the stage and site of dikaryotization which is a key factor in the pathogenic potential of the fungus. Cytological and histological studies can offer an insight into these phenomenon. We have employed a unique approach to investigate these issues based on development of monosporidial cultures. Monosporidial output of a single teliospore was employed to study the number of loci involved in the mating system. Use of a single diploid teliospore removed the confounding effect of multiple allelism. A bipolar/single locus compatibility system could be clearly seen. The four haploid genotypes which represent the meiotic products of a single teliospore (Fig.4) have been corroborated with use of molecular markers.

Table 2 Genes postulated in RILs derived from Resistant X Susceptible crosses with both heterogeneous and homogeneous inoculum systems

Cross	Year	Inoculum system	Resistance genes postulated
WH542x HD 29	2003-05	Mixture of isolates	2
		Compatible monosporidial pairs (mixed just before inoculations)	-
	2005-06	Mixture of isolates	2
		Compatible monosporidial pairs (co-cultured for 20 days)	3
	2003-05	Mixture of isolates	2
		Compatible monosporidial pairs (mixed just before inoculations)	-
WH 542 x W 485	2005-06	Mixture of isolates	2
		Compatible monosporidial pair (Co-cultured for 20 days)	3
WH 542 X ALDAN 'S'/IAS 58	2002-03	Mixure of isolates	2
	2003-04	Single isolate, P4	2
		Compatible pair of monosporida	3

It was empirically observed that these belong to two mating types as shown in Fig. 4. In another study the non infective nature of monosporidial cultures was exploited to determine the site/time of dikaryotization . The compatible monosporidial pairs were given different kinds of pre- and post- inoculation mating opportunities. The occurrence and magnitude of mating opportunity could be ascertained from the disease level. The importance of pre inoculation mating opportunities for full expression of disease potential was clearly observed.

Genetic variability between isolates of *T. indica* has been determined on the basis of phenotypic characters, pathogenecity tests, molecular markers such as isozymes and RAPD markers (Bansal *et al* .1984 a, Bonde *et al.* 1989, Sharma *et al.* 1998 a, Mishra *et al.* 2000 and Kumar *et al*

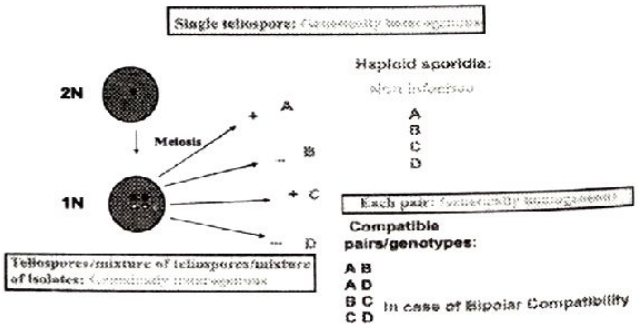

Fig. 4: Diagramatic representation: Implication of heterothallism in *Tilletia indica* on Karnal bunt resistance

2004). Variation within isolate also has great significance as from each teliospore four distinct genotypes are produced. The typical inoculum for KB screening consists of sporidial cultures derived from mixture of isolates. One can expect an astronomical number of fungal genotypes in such an inoculum. Great diversity is also expected for mating type alleles. For instance, intermating of five random monosporidial cultures revealed four mating types (Table 3). Kumar (2006) has indicated eight mating allels in monosporidia from different location of India. While distinct alleles at the single mating type locus are necessary for causing disease, the extent or level of infection seems to depend on a different set of fungal genes. Disease causing recombinants arising from a single teliospore have same constitution at the mating type locus but show differential pathogenecity.

Table 3 Cross compatiblity between monosporidia derived from Gurdaspur, Muktsar and Ludhiana isolates showing different alleles

Monosporidia With designated allel	Ear heads infected/inoculated				
	Gurdasur		Muktsar		Ludhiana
	PS 13 a_1	PS 14 a_2	M 4 a_1	M 1 a_3	L 15 a_4
PS 13 a_1		8/9	0/10	7/11	4/9
PS 14 a_1			4/10	6/11	8/10
M 4 a_2				7/10	3/8
M 10 a_3					8/10

Different pathogenic potential of compatible monosporidial pair derived from a single teliospore of P4 isolate (PS7+PS23, PS9+PS17, PS6+PS24, PS14+PS21, PS1+PS12) and Muktsar isolates (M3 +M9, M4+M10) has been recorded on 5 wheat varieties (Fig. 5).

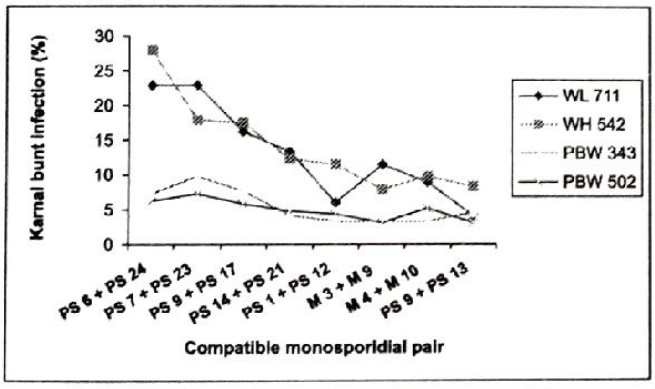

Fig. 5 : Karnal bunt infection with compatible pair of monosporidia of *Tilletia* indica showing variable pathogenecity

DEVELOPING HIGH YIELDING - KB RESISTANT WHEATS

There are certain issues related to KB screening due to which wheat breeding programmes have avoided taking up KB resistance as a regular breeding objective. Based on extensive screening work carried out by us, following critical points have emerged:

KB scores tend to vary over the crop seasons. Reliable scores would thus involve multiple screenings. An obvious question is: how many years of testing are required for a line to qualify as resistant? While there is no simple answer to this question, a minimum of three screenings have been practically adopted. The scores however, tend to stabilize within a range. For instance the KB scores of established resistance stocks like HD 29, W 485, ALDAN'S' / IAS 58, H 567.71 /3* PAR vary between 0-5% over the years.

The phenomenon of escapes in terms of individual inoculations is encountered. This makes single plant screenings, required in the segregating plant populations in a single season, less effective. One reason is improper syringing of the inoculum into the ear head: a purely mechanical matter but with serious implications. This situation is encountered because large scale inoculations in the field over a relatively short period of time necessitates dependence on unskilled field workers. A second is inability to accomplish all the inoculations before the temperature in the season rises above 25°C and third is complexity in pathogen population i.e existence of multiple mating alleles.

Further elaborate evaluation procedure against the disease involved several steps: isolation of different isolates of pathogen, their mass multiplication, inoculum preparation, syringe inoculations at appropriate growth stage and simultaneous tagging of inoculated earheads (minimum of 5 per genotype or 2-3 per plant in segregating generation), plucking the inoculated earheads followed by their threshing on line or plant basis, separately and finally counting infected and healthy grains to calculate PERCENT KB infection of each genotype or plant either by pooling the threshed grains of inoculated ear heads or individually threshing each earhead.

On account of above issues, breeding programmes have not been able to generate high level of KB resistance very effectively in high yielding backgrounds. Nevertheless, some degree of success has been achieved. At PAU Ludhiana, breeding programme was initiated as early as 1985 when crosses were attempted by using agronomically superior and KB resistant stocks. Selection for KB resistance was carried out in early segregating generations like F2 and F3. Under this programme a large number of single plants were inoculated and several agronomically good lines were generated (Sharma *et al.* 2002). Wheat variety, PBW 502 has been developed from a cross involving KB resistant parent, W 485 and high yielding varieties, PBW 343 and RAJ 1482 for cultivation in NWPZ and released in Punjab in the year,

2004 for timely sown irrigated conditions. The line showed less susceptibility to KB than the most commonly cultivated, wheat variety, PBW 343 under the coordinated testing programme. At CIMMYT, KB tolerant wheat varieties, viz; LUAN, CATBIRD, INIFAB and TOBARITO have been released for commercial cultivation in Mexico (Rajaram and Funtes-Davila 1997).

In order to develop high yielding KB resistant wheats it becomes necessary that the superior agronomic genotypes having received the resistance genes from the initially identified stocks should serve as parents in the next round of improvement. This is important as most of the identified resistant stocks are either early/late maturing with shy tillering habit and consequently low yield. Maintaining desirable levels of resistance, however, is difficult under this strategy. As an alternative we have attempted a more direct approach. The highly resistant stock such as KBRL 22 which was derived from resistant x resistant crosses as discussed previously were used as donors in a backcross programe to introgress Karnal bunt free trait into PBW 343. Simultaneously, backcrosses and inoculations were followed. Each backcross generation was advanced from Karnal bunt free plants only. The segregation pattern in these generations indicated two independently segregating, dominant genes which jointly confer KB-free behaviour (Sharma *et al.* 2003, 2004).

Yield potential of the lines derived from KBRL22/3*PBW343 was evaluated in replicated yield trials during 2003-04. These lines, had a high degree of resistance. Several of them yielded at par with PBW 343. Break down of leaf rust resistance in PBW 343 background carried by these lines however, prevented the commercial deployment of this material. In response to this situation, leaf rust resistant versions of PBW 343, carrying the *Lr24* gene, were crossed to the Karnal bunt resistant versions. Complete resistance to leaf rust on account of *Lr24* was observed to segregate in F2 in the expected monogenic fashion (Dhillon *et al.* 2006). Hardly any segregation was evident for morphological traits as both parents represented PBW 343 isolines. In F 5 generation lines homozygous resistant for both the diseases and yielding at par with PBW 343 have been identified (Table 4). High yielding, KB resistant lines showing adequate rust resistance are presently under multilocation testing.

To further this strategy, resistance from diverse donors has been transferred to high yielding commercial genotypes such as PBW 343 and WH 542 to generate a large number of KB resistant isogenic materials with different resistance genes. To develop this material rapidly, the off-season was used to advance the backcross generations while at main location both backcrossing and selection for KB resistance is being performed. Now we have the prospects of using these agronomically superior lines as sources of resistance. Combining diverse KB resistance genes in high yielding backgrounds to generate highly resistant materials would also be facilitated. In a parallel development specific rust resistance genes are being pyramided in high yielding wheats using molecular markers. These materials perfectly complement the KB resistant isolines as parents for generating high yielding, multiple disease resistant varieties. To minimize the risk of dilution of KB resistance and to avoid extensive screening against KB some backcrosses may be made .using the KB resistant high yielding line as recurrent parent.

Table 4 Yield potential and rust reaction of selected Karnal bunt resistant lines in PBW 343 background from different trials conducted in 2006-07

KBLR Lines No.	Yield (kg/plot)	Rust Score		KB (%)	*Lr 24* marker
		YR	LR		
		Trial 1			
3	3.19	TS	TS	0.00	P*
24	3.34	5S	20S	2.10	A*
PBW 343	3.41	20S	40S	11.5	A
PBW 502	3.37	20S	40S	4.8	A
PBW 550	3.18	0	0	28.8	A
		Trial 2			
33	3.24	10S	0	0.0	P
41	3.30	5S	20S	0.0	P
42	3.29	5S	20S	0.0	P
43	3.35	5S	20S	0.0	P
50	3.15	5S	20S	0.0	P
59	3.48	10S	TS	0.0	P
PBW 343	3.00	30S	40S	10.5	A
PBW 502	3.63	10S	40S	4.0	A
PBW 550	3.11	10S	0	26.5	A

Trial 3

60	3.10	5S	0	0.250	P
65	3.41	0	0	0.00	P
PBW 343	3.60	20S	40S	12.6	A

*P indicates presence, A indicates absence

CHROMOSOMAL LOCATION AND TAGGING OF KB RESISTANCE GENES

Analysis of monosomic series, aneuploids, ditelosomics and nullitetra compensat- ing groups involving D-genome of wheat variety, Chinese Spring and addition and substitution lines of D-genome in the background of *T. durum* var. *Langdon* (Gill *et al.* 1988; Singh, 1989) had shown that 5 chromosomes (1D, 2D, 3B, 5B and 7A) were governing to KB resistance / susceptibility.

Based on the restriction fragment length polymorphism (RFLP) of RILs derived from a cross between a resistant synthetic wheat (*T. turgidum* 'ALTAR 84 x *T. tauschi*) and the susceptible common wheat cultivar, Opata 85, regions on chromosome arms 3 BS and 5AL were shown to carry marker alleles (from Altar durum parent) which were consistently associated with the reduced disease (Nelson *et al.* 1998).

KB resistance genes from *T. monococcum* which were first brought into the synthetic hexaploid backgrounds (*T. turgidum* x *T. monococcum*) and then transferred into WL 711 were tagged with microsattelite markers. Markers, gwm 382, gwm 369, gwm 637, gwm156 and gwm 617 which mapped on 2AS, 3AS, 4AL, 5AL and 6AL chromosomes respectively, were found to be associated with KB resistance (Vasu *et al.* 2000). In an Indo-Swiss project, a QTL has been detected on chromosome 1A linked with Xcfa 2158 as left flanking markers with R2 value of 32 % in a RIL derived from *T. monococcum* - Acc. 14087 x *T. boeoticum*-Acc. 5088 (Singh *et al.* 2004).

On the basis of RFLP and AFLP mapping in segregating populations from HD 29 x WL 711, KB resistance gene(s) were observed to be located on chromosome groups 4BL and 7 BS of wheat (Singh *et al.* 1994, 1999). In a follow up study (Sukhwinder Singh 2003) based on a RIL population from the same cross 90 SSRs and 81 AFLP loci were mapped. Markers on chromosome 2A, 4A and 7B accounted collectively for about 1/3rd of the variation. The genomic region of largest effect identified on long arm of 4B accounted for 25% of phenotypic variation for KB resistance. Continuing the studies further QTLs associated with resistance to KB were identified in two recombinant inbred mapping populations from WH542/HD29 and WH542/W485. Two new QTLs (Qkb.ksu-5BL.I and Qkb.ksu-6BS.I) with resistance alleles from HD 29 were identified and mapped in the intervals Xgdm116-Xwmc235 on chromosome 5B (deletion bin 5BL9-0.76-0.79) and Xwmc105-Wgwm88 (C-6BS5-0.76) on chromosome 6B. They explained up to 19 and 13 % phenotypic variance, respectively. Another QTL (Qkb. ksu-4BL.I) with resistance allele from W 485 mapped in the interval (Xgwm6- Xwmc349) on chromosome 4B(4BL5-0.86-1.00) and explained up to 15 % of phenotypic variance. Qkb.ksu-6BS.I showed pairwise interactions with loci on chromosomes 3B and 6A. Markers suitable for marker assisted selection are available for all three QTLs.

Extent of disease severity in the artificially inoculated kernels is environment dependent and is also influenced by the pathogenecity alleles of various compatible types which are many. Therefore, year to year variation exists in the disease score when inoculations are carried out under field conditions. Marker assisted selection might help in large scale selection of segregating populations for KB resistance.

CONCLUSION

Understanding pathogen population is necessary for exploiting resistance, more so when dealing with a heterothallic fungus. Viewing variability for pathogenecity even within recombinants derived from a single teliospore and presence of multiple alleles for pathogenecity in different isolates, using mixture of pathogen population continues to be the best strategy for identifying resistance, breeding for high yielding disease resistant wheat varieties and even for tagging of genes.

REFERENCES

Aujla S S, Grewal A S, Gill K S and Sharma I. 1980. A screening technique for Karnal Bunt disease of wheat. *Crop Improvement* **7**: 145-46

Aujla S S, Grewal A S, Gill K S and Sharma I. 1982. Artificial creation of Karnal bunt disease of wheat. *Cereal Research Communication* **10**:171- 176.

Aujla S S and Sharma I. 1990. Evaluation of fungitoxicants against Karnal bunt disease of wheat. *Journal of Research Punjab Agricultural University, Ludhiana* **27**(3) : 434-436.

Aujla S S, Sharma I and Gill K.S. 1990. Sources of resistance in wheat to Karnal bunt. *Indian Phytopathology* **43** (1) : 92-93.

Aujla S S, Sharma I and Gill K S. 1992. Stable resistance in wheat to Karnal bunt (*Tilletia indica*). *Ind J agric Sciences* **62** : 171-172

Aujla S S, Sharma I, Gill K S and Grewal A S. 1985. Variable resistance in wheat germplasm to Neovossia indica. In: 3rd National Seminar Genet Wheat Improvement held at IARI Reg Res Station, Flowerdale Shimla. Himachal Pradesh, India

Aujla S S, Grewal A S and Sharma I. 1983. Yield and vigour components of wheat as affected by Karnal bunt pathogen (*Neovossia indica*) *J Res Punjab Agric Univ.* **20**: 231-35.

Bag T K, Singh D V and Tomar S M S.1999. Inheritance of resistance to Karnal bunt (*Tilletia indica* Mitra) in some Indian bread wheat (*Triticum aestivum* L.) lines and cultivars. *Journal of genetics and Plant Breeding* **53**: 67-72.

Bansal R, Singh D V and Joshi L M. 1984. Comparative morphological studies in teliospores of *Neovossia indica*. *Indian Phytopathology* **37**: 355-357.

Bedi K S, Sikka M R and Mundkur B B. 1949. Transmission of wheat bunt due to *Neovossia indica* (Mitra) Mundkur. *Indian Phytopathology* **2**: 20-26.

Bonde M R, Peterson G L and Matsumoto T T. 1989. The use of isozymes to identify teliospores of *Tilletia indica*. *Phytopathology* **79**: 596-99.

Bonde M R, Peterson G L, Funtes D G, Aujla S S, Nanda G S and Philips J G. 1996. Comparison of the virulence of isolates of *Tilletia indica*, causal agent of Karnal bunt of wheat. *Plant Disease* **80**: 1071-1074.

Boucher C. 2002. Karnal bunt incidence in South Africa. XIII Biennial Workshop on smut fungi held at Dallas, Texas from June 16-19, 2002.

Carris M. Loris, Castelbury Lisa A and Goates J. 2006. Non systemic bunt fungi – *Tilletia indica* and *Tilletia horrida*: A review of history, systematics and Biology. *Annual Review Phytopathology* **44:** 5.1-5.21.

Chand K, Gill K S, Nanda G S and Singh G. 1989. Breeding for Karnal bunt resistance through intermating of wheat cultivars with low coefficient of infection. *Crop Improvement* **16** : 178-179.

Chona B L, Munjal R L and Adlakha K L.1961. A method for screening wheat plants for resistance to *Neovossia indica*. *Indian Phytopathology* **14** : 99-101

Chhuneja P, Kaur S and Dhaliwal H S. 2004. Screening of US winter wheats under a screen house with simulated epidemiological parameters for high incidence of Karnal bunt. XIVth Biennial Workshop on Smut Fungi held at Idaho, USA from June 23rd – 25th, 2004.

Crous P W, Van Vaarsveld A B, Castelebury Carris M and Pretorius Z B. 2000. Karnal bunt found in South Africa http: // www sassp.co.29/x6/ new disease reports, January, KB htm.

Datta R, Singh H, Gupta V S, Ranjekar P K, and Dhaliwal H S.1999. Gene – for- gene relationship for resistance in wheat to isolates of Karnal bunt, *Neovossia indica*. *Plant Breeding* **118**: 362-364

Dowell F E, Boratynski T N, Ykema R E, Dowdy A K and Staten R T. 2002. Use of optical sorting to detect wheat kernels infected with *T. indica*. *Plant Disease* **86**: 1011-1013

Duran R. and Cromarty R.1977. *Tilletia indica* : a heterothallic wheat bunt fungus with multiple alleles controlling incompatibility. *Phytopathology* **67** : 812-815

Fuentes - Davila G. 1997. Karnal bunt of wheat. In: Bunts and Smuts of Wheat - An international Symposium held at Raleigh, North Carolina and organized by North American Plant Protection Organization, Ottawa, Canada, Aug 17-20,1997.

Fuentes-Davila G and Rajaram S.1994. Sources of resistance to *Tilletia indica* in wheat. *Genetics* **50**: 205-09.

Fuentes - Davila G, Rajaram S and Singh G. 1995. Inheritance of resistance to karnal bunt (*Tilletia indica* Mitra) in bread wheat (*Triticum aestivum* L.). *Plant Breeding* **114**: 250-252.

Garrett K A and Bowden R L. 2002. An allee effect reduces the invasive potential of *Tilletia indica*. *Phytopathology* **92**: 1152-59.

Gill K S, Randhawa A S, Aujla S S, Dhaliwal H S, Grewal A S and Sharma I. 1981. Breeding wheat varieties resistant to Karnal bunt. *Crop Improvement* **8**: 73-80.

Gill K S, Dhaliwal H S and Multani D S. 1988. Synthesis and evaluation of *T. durum* and *T. monococcum* amphiploids. *Theor. Appl. Genet.* **75**: 912-916

Gill K S, Nanda G S, Singh G, Chand K, Aujla S S and Sharma I.1990. Study of gene effects for Karnal bunt (*Neovossia indica* Mitra) resistance in bread wheat. *Indian Journal of Genetics* **50** : 205-209

Gill K S, Sharma I and Aujla S S. 1993. Karnal bunt and wheat production. Journal Punjab Agricultural University, Ludhiana, India, pp. 153

Goates J Blair. 2004. Susceptibility of wheat to *T. indica* during stages of plant development. XIVth Biennial Workshop on Smut Fungi held at Idaho, USA from June 23rd – 25th, 2004.

Goates J Blair and Funtes D G. 2002. Reaction of US spring wheat cultivars to karnal bunt. XIII Biennial Workshop on smut fungi held at Dallas, Texas from June 16-19, 2002

Grewal T S, Aujla S S and Sharma I. 1996. Karnal bunt resistance in wheat – genetic studies. *Plant Disease Research* **11**: 35-43

Harjit Singh, Grewal T. S., Pannu P. P. S. and Dhaliwal H. S. 1999. Genetics of resistance to Karnal bunt disease of wheat. *Euphytica* **105**: 125-131

Hayman B L.1954. The analysis of variance of diallel tables. *Biometrica* **10**: 233-44.

Howard and Howard. 1909. Wheat in India. I. Production, varieties and improvement. pp 281. Thacker Spink and Co, Calcutta.

Holton C. S.1949. Observations on *Neovossia indica*. *Indian Phytopathology* **2**: 1-5.

Jinks J L. 1954. The analysis of continuous variation in a diallel cross of *Nicotiana rustica* varieties. *Genetics* **19**: 769-88.

Kumar M, Luthra O P, Chawla V and Arya R. 2002. Genetics of resistance to karnal bunt of wheat { *Neovossia indica* (Mitra) Mundkur} *Indian Journal Genetics* **62** (2): 113-115.

Kumar J, Saharan M S, Sharma A K, Sharma S, Somvir and Nagarajan S. 2004. Pathogenic and molecular variation among Indian isolates of *Tilletia indica* causing Karnal bunt of wheat. *Indian Phytopathology* **57**:144-49.

Kumar J. 2006. DWR Annual Report, 2006-07.

Krishna A and Singh R A. 1982. Taxonomy of Karnal bunt fungus: Evidence in support of genus *Neovossia. Indian Phytopathology* **35**: 544-45.

Krishna A and Singh R A.1983. Multiple alleles controlling the incompatibility in *Neovossia indica. Indian Phytopathology* **36**: 746-48.

Locke C M and Watson A J. 1975. Foreign plant diseases intercepted in quarantine inspection. *Plant Disease Reporter* **39** (6):518.

Mishra A, Kumar A, Garg G K and Sharma I (2000) Determination of genetic variability among isolates of *Tilletia indica* using Random Amplified polymorphic DNA analysis. *Plant Cell Biotechnolgy Molecular Biology* **1**: 29-36.

Mitra M.1931. A new bunt on wheat in India. *Annals of Applied Biology* **18** : 178-179

Morgunov A, Montana J and Rajaram S. 1994. Genetic analysis of resistance to karnal bunt (*Tilletia indica* Mitra) in bread wheat. *Euphytica* **74**: 41-46.

Munjal R L. 1970. Studies on Karnal bunt of wheat. Ph.D. Thesis, Panjab University, Chandigarh, India.

Munjal R L. 1971. Epidemiology and control of Karnal bunt of wheat. Proc Epidemiology, forecasting and control of Plant Disease, Symp. INSA, New Delhi (abstr.).

Nanda G S, Chand K, Sohu V S and Sharma I. 1995. Genetic analysis of karnal bunt resistance in wheat. *Crop Improvement* **22** : 189-93.

Nelson J C, Atrique J E, Fuentes D G and Sorrells M E. 1998. Chromosomal location of genes for resistance to Karnal bunt in wheat. *Crop Science* **38** : 231-236.

Pedro Figueroa-Lopez, Guillermo Fuentes-Davila and Miguel A. Camacho-Casas. 2004. Relationship between Karnal bunt infection obtained from wheat lines artificially inoculated with and without sprinkler-mist irrigation. XIVth Biennial Workshop on Smut Fungi held at Idaho, USA from June 23rd – 25th, 2004.

Perring T M, Adam C J, Siebert J M, Holmes G J, Jackson L and Kallenback R L. 1996. Likelihood of karnal bunt spread from wheat harvested in the Imperial and Palo Verde Valleys of California. Report : Institute for Desert Agriculture, University of California : 89.

Rajaram S and Fuentes-Davila G.1997. Development of wheat varieties with resistance to Karnal bunt. In: Vedpal S Malik and D E Mathre (eds). Bunts and Smuts of wheat: An International Symposium 305-317.

Royer M H and Rytter J. 1985. Artificial inoculation of wheat with *Tilletia indica* from Mexico and India. *Plant Disease* **69**: 317-19.

Sharma I, Nanda G S and Kaloty P K. 1998. Variability in Neovossia indica: based on pathogencity and isozyme analysis. *Tropical agricultural Research Extension* **1**: 159-61.

Sharma S K, Dhaliwal H S, Bagga P S, Randhawa A S and Pal S S.1991. Gene action for Karnal bunt resistance in wheat. *Indian Journal Genetics* **57**: 374-75.

Sharma I, Nanda G. S. and Chand K. 1997. Karnal bunt resistance in bread wheat. In: Proceedings 3rd Agricultural Sciences Congress (NAAS) held at PAU from March 12-15, 2 : 147.

Sharma I, Nanda G S and Chand K. 2001. Three karnal bunt resistant wheat genetic stocks registered. *Indian Phytopathology* **54** (3) : 402.

Sharma I, Nanda G S, Chand K and Sohu V S. 2002. Status of Karnal bunt (*Tilletia indica*) resistance in Bread wheat (*Triticum aestivum*). *Indian Journal of Agricultural Sciences* **71**(12): 794 –6.

Sharma I, Bains N S and Nanda G S. 2004. Inheritance of Karnal bunt – free trait in bread wheat. *Plant Breeding* **123**: 96-97.

Sharma I, Bains N S, Nanda G S and Satija D R. 2003. Incorporation of Karnal bunt resistance in wheat varieties PBW 343. *Crop Improvement* **30**: 21-24.

Sharma I, Bains N S, Singh K and Nanda G S. 2005 a. Additive genes at nine loci govern Karnal bunt resistance in a set of common wheat cultivars. *Euphytica* **142**: 301-07.

Sharma M, Nanda G S, Sharma I and Sohu V S. 2001. Inheritance of resistance to karnal bunt (*Tilletia indica* Mitra) in bread wheat (*Triticum aestivum* L.). *Crop Improvement* **28** (2): 207-213.

Sharma M, Nanda G S, Sharma I and Sohu V S. 2004. Qualitative approach to study karnal bunt inheritance in bread wheat (*Triticum aestivum* L.). *Journal Research PunjabAgricultural University* **41**: 1-7.

Sharma R C. 2001. Technical bulletin: Karnal bunt, *PunjabAgricultural University.*

Singh D, Singh R, Rao V L M, Karwasra S S and Beniwal M S. 1996. Relation between weather parameters and Karnal bunt in wheat. *Indian Journal of Agricultural Sciences* **56**: 522-25.

Singh D V, Agarwal R, Shareshtha J K, Thappa B R, Dubin H J. 1989. First report of *Neovossia indica* on wheat in Nepal. *Plant Disease* **73**: 277.

Singh G, Rajaram S, Montoya J and Fuentes - Davila G. 1995a. Genetic analysis of resistance to Karnal bunt in bread wheat. *Euphytica* **81**: 117-120.

Singh G, Rajaram S, Montoya J and Fuentes Davila G. 1995 b. Genetic analysis of karnal bunt resistance in 14 Mexican bread wheat genotypes. *Plant Breeding* **114**: 439-441.

Singh K.1989. Cytogenetic analysis of Karnal bunt resistance involving wild donors and cultivated species of wheat. Ph.D. Thesis, Punjab Agricultural University, Ludhiana, Punjab, India.

Singh K, Chhuneja P, Kaur S, Kaur A and Dhaliwal H S.2004. Molecular mapping of Karnal bunt resistance in *Triticum monococcum* and its transfer to tetraploid and hexaploid wheat XIVth Biennial Workshop on Smut Fungi held at Idaho, USA from June 23rd – 25th, 2004: 15.

Singh S, Gill K S, Dhaliwal H S, Harjit S and Gill B S.1994. Towards molecular tagging of karnal bunt resistant genes in wheat. *Journal of Plant Biochemistry and Biotechnology* **3**:79-83.

Singh S, Grewal T S, Sodhi M and Dhaliwal H S.1999. Identification of amplified fragment length polymorphism markers associated with Karnal bunt (*Neovossia indica*) resistance in bread wheat. *Indian J. Agric. Sci.* **69**: 497-501.

Sukhwinder-Singh, Brown-Guedira G L, Grewal T S, Dhaliwal H S, Nelson J C, Singh H, Gill B S. 2003. Mapping of a resistance gene effective against Karnal bunt pathogen of wheat. *Theoretical Applied Genetics* **106** : 287-292.

Sukhwinder-Singh, Sharma I, Bains N S, Brooks S A, Brown-Guedira G L, Grewal T S, Dhaliwal H S, Nelson J C, Singh H, Gill B S. 2004. Molecular characterization of karnal bunt resistance in wheat XIVth Biennial Workshop on Smut Fungi held at Idaho, USA from June 23 – 25, 2004: 12.

Sukhwinder-Singh , Sharma I, Sehgal S K, Bains N S, Guo Z, Nelson J C and Bowden R L. 2007. Molecular mapping of QTLs for Karnal bunt resistance in two recombinant inbred populations of bread wheat. Theoretical and Applied Genetics (in press).

Torabi M, Mardoukhi V, Jaliani N.1996. First report on the occurrence of partial bunt on wheat in the Southern parts of Iran. *Seed and Plant* **12**: 8-9.

Vasu K, Singh H, Chhnuja P, Singh S and Dhaliwal H S. 2000. Molecular tagging of karnal bunt resistance genes of *Triticum monococcum* L. transferred to *Triticum aestivum* L. *Crop Improvement* **27**(1) : 33-43.

Villareal R L, Mujeeb-Kazi A, Fuentes D G and Rajaram S. 1995. Inheritance of resistance to *Tilletia indica* (Mitra) in synthetic hexaploid wheat x *Triticum aestivum* cross. *Plant Breeding* **114**: 547-548.

Villareal R L, Mujeeb-Kazi A, Fuentes D G and Rajaram S. 1996. Registration of four synthetic hexaploid wheat germplasm lines derived from *Triticum turgidum* x *T. tauschii* crosses and resistance to Karnal bunt. *Crop Science* **36** : 218.

Villareal R. L, Mujeeb-Kazi A, Fuentes D G and Rajaram S. and E.Del Toro. 1994. Resistance to Karnal bunt in synthetic hexaploid wheat derived from *Triticum turgidum* x *T. tauschii. PlantBreeding* **112**:63-69.

Warham E J.1986. Karnal bunt disease of wheat – a literature review. *Tropical Pest Management* **32**: 229-242.

Warham E J. 1988. Screening for Karnal bunt (*Tilletia indica*) resistance in wheat, Triticale, Rye and Barley. *Canadian Journal of Plant Pathology* **10**: 57-60.

Warham E J. 1990. A comparison of inoculation techniques for assessment of germplasm susceptibility to Karnal bunt (*Tilletia indica*) disease of wheat. *Annals of Applied Biology* **116**:43-60.

Williams P C.1983. Incidence of stinking smut (*Tilletia* spp.) on commercial wheat samples in Northern Syria. Rachis (ICARDA) Barley, Wheat and Triticale Newsletter 2:21.

Ykema R E, Floyd J P, Palm M E, Peterson G L 1996. First report of Karnal bunt of wheat in the United States. *Plant Disease* **80**: 120.