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Resistance to Karnal Bunt (*Tilletia indica* Mitra) in Synthetic Hexaploid Wheats Derived from *Triticum turgidum* × *T. tauschii*

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With 4 tables

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Abstract

Synthetic hexaploids (SH) developed at the International Maize and Wheat Improvement Center (CIMMYT), involving four *Triticum turgidum* and nine *T. tauschii* parents, were evaluated for resistance to Karnal bunt (KB) (*Tilletia indica* Mitra) during three crop seasons over three years at Ciudad Obregon, Sonora, Mexico. Ten tillers of each SH at boot stage, taken at random, were injected with a suspension of sporidia in water (10,000 spores/ml of water). At maturity the inoculated spikes were threshed individually and evaluated for the percentage KB-infected grains. Based on the mean KB score of each entry for three seasons, 49 % of the SH were immune (0 % infection) to KB. Highly resistant expressions characterized the SH which appeared to be influenced by the resistance of their *T. turgidum* and/or *T. tauschii* parents. The overall mean infection of the SH wheats was 0.24 % compared to 56.14 % in the susceptible bread wheat check cultivat 'WL711'. Transfer of KB resistance genes from SH wheats into bread wheat is currently underway at CIMMYT.

Key words: *Triticum turgidum* — *T. tauschii* — *T. aestivum* — synthetic hexaploid (SH) — *Tilletia indica* — Karnal bunt (KB) — artificial inoculation — resistance — genetic variability.

Karnal bunt (KB) of wheat is caused by *Tilletia indica* Mitra (Synonym *Neovossia indica* [Mitra] Mundkur), a fungus that infects wheat (Mitra 1931) and triticale (Agarwal et al. 1977) during flowering (Bedi et al. 1949). The disease occurs in India (Mitra 1931), Mexico (Duran 1972), Iraq (CMI 1974), Pakistan (Munjal 1975), and Nepal (Singh et al. 1989). In Mexico, KB was first reported by Duran (1972). During the early 1970s it was also found in the Yaqui and Mayo Valleys of the northwestern state of Sonora. Twenty years later, the disease had spread south into the neighbouring state of Sinaloa. It has now spread further to Baja California Sur, but not to northern Sonora or to Baja California Norte (Lira 1984, Prescott 1984, Warham 1986, Brennan et al. 1990).

Estimates of loss due to KB are complex for two reasons: (i) it affects quality rather than yield, and (ii) since it is a seed-borne disease to which zero tolerance is required by many countries, indirect costs are incurred in preventing its spread. In Mexico, during a season with severe disease infection such as 1984/85, 68.3 % of grain delivered to seed depots had KB infection. Of this, 5.9 % had more than 3 % infection. In 1987/88 when the disease incidence was low, 3 % of the grain had some infection, but none of the samples had more than 2 % infection. The indirect and direct economic losses caused by KB in northwestern Mexico are US $ 7.0 million per year, indicating that effective measures to control the disease could result in substantial savings (Brennan et al. 1990).

Because KB is widespread in farmers' fields in northwest Mexico, few options are available to farmers to reduce costs incurred in controlling this disease. One major possibility is the

development of cultivars with genetic resistance. High levels of resistance to the disease has been reported in durum wheat, triticale and several *Aegilops* (Ae.) species which can serve as sources of resistance (BEDI et al. 1949, GAUTAM et al. 1977, GILL et al. 1981, WARHAM et al. 1986, FUENTES-DAVILA et al. 1992). Resistance is generally lacking in common wheats (*Triticum aestivum* L.).

GILL et al. (1981) proposed that resistance should be sought in *Ae. squarrosa* (*Triticum tauschii;* 2n = 2x = 14, DD) because group 6 chromosomes are involved in the reaction to KB, with an apparently large influence of the D genome. When GILL et al. (1983) compared KB incidence among aneuploids of *T. turgidum* and *T. aestivum,* they found higher levels of susceptibility in those materials where either chromosome 1A or 1D were missing, thus suggesting that chromosomes 1A and 1D might have a complementary gene system for KB resistance. Moreover, the long arm of chromosome 4D appeared to possess a dominant gene controlling susceptibility (GILL et al. 1983).

Accessions of *Ae. biuncalis, Ae. columnaris, Ae. crassa, Ae. juvenalis, Ae. ovata, Ae. speltoides, Ae. umbellulata, Ae. variabilis, Ae. vavilovi, Ae. ventricosa* and *Ae. squarrosa* were subsequently reported to be resistant to KB using boot inoculation under greenhouse conditions (WARHAM et al. 1986). Interspecific hybrids between these species and *T. turgidum* and *T. aestivum* have been produced (KIMBER and FELDMAN 1987, VILLAREAL et al. 1991). The *T. turgidum* × *Ae. squarrosa* synthetic hexaploid (SH) wheats developed at CIMMYT have shown more promise in increasing the genetic variability in a wheat breeding programme because of the genetic proximity of the D genome of *Ae. squarrosa* to *T. aestivum* (MUJEEB-KAZI et al. 1993).

The object of this study was to evaluate the resistance of SH wheats derived from crosses using *T. turgidum* and *T. tauschii* parents highly resistant or immune to KB (*Tilletia indica*) and using artificial inoculation in the field.

Materials and Methods

Forty-five SH (2n = 6x = 42; AABBDD) derived from ten crosses involving four high-yielding CIMMYT *T. turgidum* parents and nine *T. tauschii* accessions selected from the CIMMYT Germplasm Bank were included. The durum wheat and *T. tauschii* backgrounds of the test materials are shown in Tables 1 to 4. The *T. tauschii* identification numbers (Tables 1—4) correspond to their respective Interspecific Vernalized (Inter-Ver) alien species registration numbers at CIMMYT. *T. aestivum* cultivar 'WL711' from India was included as a susceptible check in both greenhouse and field tests.

Teliospores from various locations in the Yaqui Valley, Sonora, Mexico were used to insure a genetically heterogenous composite of the fungus population. To isolate teliospores, infected kernels were shaken in a water-tween-20 solution for 15 s, centrifuged at 3,000 rpm, and sieved using a 60 micron mesh to remove the kernel residue. Thereafter, they were surface-sterilized with 0.5 % sodium hypochlorite while centrifuging for about 2 min, rinsed in sterile distilled water, plated on 1.5 % water agar and incubated at room temperature. After 5—8 days, germinating teliospores were transferred to potato-dextrose-agar (PDA) to which sterile water was added. Nine days later fungal colonies were scraped and further inoculated on to additional PDA plates. After 8—10 days, the PDA fungus colony was cut into small pieces and placed on to the lids of sterile, glass petri plates. This process enhances the release of many secondary sporidia from the fungal colonies. A small amount of sterile water was added to the bottom of each. Then the allantoid sporidia were counted every 24 h using a haemocytometer, and the spore concentration adjusted to 10,000/ml. Ten tillers taken at random from each entry were inoculated during the boot stage, stages 48—49 according to ZADOKS et al. (1974), by injecting 1 ml/tiller of the sporidial suspension with a hypodermic syringe. Tillers were tagged with colour coding tape to indicate the date of inoculation.

The *T. tauschii* accessions were inoculated separately because of their vernalization requirement. One-week old seedlings of the *T. tauschii* accessions were vernalized in a growth chamber for 8 weeks at 8 °C, 70 % relative humidity, and 8 h light (fluorescent and incandescent light with approximately 20,000 lux). Following vernalization, seedlings of the materials were transplanted into pots and grown in the greenhouse at El Batan, Mexico. The mean environmental growth regimes were 17.6 to 22.8 °C, 14 h natural light and approximately 60 % relative humidity. The plants were artificially inoculated with the KB pathogen using the boot injection technique described above. At maturity, 10 spikes were graded for infection and the overall percentage infection calculated for each accession.

The durum cultivars and the SH were grown in the irrigated fields of the Mexican Institute of Forestry, Agriculture, and Livestock, Campo Agricola Experimental del Valle de Yaqui research station, Sonora, Mexico (27°20′ N, 105°55 W, elevation 39 m

above sea level) during the 1988—89, 1989—90 and 1991—92 wheat seasons. They were grown in 2-m long, two-row plots on 90 cm wide beds with 20 cm between rows using a Completely Randomized Design with two replications. The boot injection inoculation technique was employed. Overhead sprinklers with fine nozzles were used to achieve the optimum relative humidity needed for successful disease infection. After inoculation, sprinklers were turned on 3—5 times daily for 8 min each time for 12 days. At maturity, 10 spikes from each entry and the susceptible check, were harvested and hand threshed to determine the percentage of kernels infected with KB. Due to the tightness of the glumes of the synthetic lines, their spikes were placed between wet blotters for 1 h to facilitate threshing.

The mean KB infection levels of the test entries across years were pooled and subjected to an analysis of variance procedure following square-root transformation. Mean separation of the infection scores of the synthetics and their respective progenitors was conducted using the Least Significant Difference (LSD) in the transformed scale. However, untransformed mean KB infection scores were utilized for discussion purposes.

Results

The overall mean KB infection levels of the *T. turgidum* and *T. tauschii* parents, and the 45 SH are presented in Tables 1 to 4. Durum cultivars 'Altar' and 'Laru' showed 0.84 % and 0.36 % infection; whereas 'Chen' and 'Duergand' showed 0 % infection during the three years. Similarly, *T. tauschii* accessions 192, 211, 214, 219, 224, and 309 exhibited 0 % infection in the greenhouse compared with 78 % infection in the susceptible control *T. aestivum* cultivar 'WL711'. *T. tauschii* accessions 198, 205, and 221 had very low infection levels.

Three cycles of KB screening of SH lines had KB infection ranging from 0 % to 1.82 %. The overall mean KB infection of the synthetics was 0.24 % compared to 56.14 % of the susceptible *T. aestivum* check cultivar 'WL711'. Forty-nine percent of the synthetics tested showed an immune response (0 %) to *Tilletia indica*.

Table 1. Mean Karnal bunt (KB) infection of synthetic hexaploids (SH) derived from crosses of *Triticum turgidum* cv. 'Altar' with five *T. tauschii* accessions (Inter-Ver)

Parents and SH	Entry code	% KB infection	Trans. mean	LSD** (0.05)
Altar 84	Altar	0.84	1.15	
T. tauschii 192	192	0	0.71	
T. tauschii 198	198	0.14	0.80	
T. tauschii 211	211	0	0.71	
T. tauschii 219	219	0	0.71	
T. tauschii 224	224	0	0.71	
Synthetic hexaploids				
Altar/192	A192a*	0.22	0.83	0.26
Altar/198	A198a	0	0.71	0.10
Altar/198	A198b	0	0.71	
Altar/198	A198c	0	0.71	
Altar/211	A211a	0	0.71	0.16
Altar/219	A219a	0.07	0.75	0.93
Altar/219	A219b	1.82	1.52	
Altar/219	A219c	0.42	0.96	
Altar/224	A224a	0.08	0.76	0.11
Altar/224	A224b	0	0.71	
Altar/224	A224c	0	0.71	
Altar/224	A224d	0.09	0.77	

* The alphabetic suffix for each code indicates the lines in each derived synthetic.
** LSD values used to compare parents and SH of each cross.

Table 2. Mean Karnal bunt (KB) infection of synthetic hexaploids (SH) derived from crosses of *Triticum turgidum* cv. 'Chen' with two *T. tauschii* accessions (Inter-Ver)

Parents and SH	Entry code	% KB infection	Trans. mean	LSD** (0.05)
Chen	Chen	0	0.71	
T. tauschii 205	205	0.04	0.73	
T. tauschii 224	224	0	0.71	
Synthetic hexaploids				
Chen/205	C205a*	0.36	0.93	0.32
Chen/205	C205b	0.45	0.97	
Chen/205	C205c	0.10	0.77	
Chen/205	C205d	0	0.71	
Chen/205	C205e	0.16	0.81	
Chen/205	C205f	0.53	1.01	
Chen/205	C205g	0.32	0.90	
Chen/205	C205h	0	0.71	
Chen/205	C205i	0.14	0.80	
Chen/205	C205j	0	0.71	
Chen/224	C224a	0	0.71	0.04
Chen/224	C224b	0	0.71	
Chen/224	C224c	0	0.71	
Chen/224	C224d	0	0.71	
Chen/224	C224e	0	0.71	
Chen/224	C224f	0	0.71	
Chen/224	C224g	0	0.71	
Chen/224	C224h	0.08	0.76	
Chen/224	C224i	0	0.71	
Chen/224	C224j	0	0.71	
Chen/224	C224k	0	0.71	

* The alphabetic suffix for each code indicates the lines in each derived synthetic.
** LSD values used to compare parents and SH of each cross.

Table 3. Mean Karnal bunt (KB) infection of synthetic hexaploids (SH) derived from crosses of *Triticum turgidum* cv. 'Duergand' with two *T. tauschii* accessions (Inter-Ver)

Parents and SH	Entry code	% KB infection	Trans. mean	LSD** (0.05)
Duergand	Duerd	0	0.71	
T. tauschii 214	214	0	0.71	
T. tauschii 221	221	0.06	0.75	
Synthetic hexaploids				
Duerd/214	D214a*	0	0.71	0.70
Duerd/214	D214b	0.28	0.88	
Duerd/214	D214c	1.44	1.39	
Duerd/214	D214d	0.66	1.08	
Duerd/221	D221a	0.58	1.04	0.35
Duerd/221	D221b	0	0.71	
Duerd/221	D221c	0.22	0.85	
Duerd/221	D221d	0	0.71	

* The alphabetic suffix for each code indicates the lines in each derived synthetic.
** LSD values used to compare parents and SH of each cross.

Table 4. Mean Karnal bunt (KB) infection of synthetic hexaploids (SH) derived from the cross of *Triticum turgidum* cv. 'Laru' with one *T. tauschii* accession (Inter-Ver)

Parents and SH	Entry code	% KB infection	Trans. mean	LSD** (0.05)
Laru	Laru	0.36	0.93	
T. tauschii 309	309	0	0.71	
Synthetic hexaploids				
Laru/309	L309a*	1.70	1.48	0.36
Laru/309	L309b	1.04	1.24	
Laru/309	L309c	0.26	0.87	
Laru/309	L309d	0.36	0.93	

* The alphabetic suffix for each code indicates the lines in each derived synthetic.
** LSD values used to compare parents and SH of each cross.

Infection levels of 12 advanced SH lines derived from five crosses involving 'Altar' and five *T. tauschii* accessions were comparable to their *T. tauschii* parent and significantly ($P < 0.05$) lower than their durum parent (Table 1). Several SH lines derived from the *T. tauschii* accessions 198, 211 and 224, were immune and therefore similar to the reaction of the *T. tauschii* parent. Other derived lines such as A192a, A219a, A219c, A224a and A224d were intermediate in their reaction between the *T. tauschii* and *T. turgidum* parent.

Derivatives from the 'Chen' × 205 cross, gave different degrees of disease expressions that were not, however, statistically different ($P > 0.05$; Table 2). Almost all the 'Chen' × 224 derived lines gave immune responses to KB except line C224h which gave 0.08 % infection. The resistance expression of all these lines was consistent with that of the parents ($P > 0.05$).

The four derivatives from the 'Duergand' × 214 cross gave infection scores comparable to both parents ($P > 0.05$) as did the derivatives from 'Duergand' × 221 (Table 3). Similarly the resistances expressed by the synthetic lines derived from each of the crosses were not significantly different from each other ($P > 0.05$).

From the four 'Laru' × 309 cross derivatives, lines L309c and L309d showed resistance comparable to their parents ($P > 0.05$; Table 4).

Discussion

Based on the mean KB score of each entry for three seasons, SH wheats showed highly resistant to immune (0 % infection) reactions to *Tilletia indica*. Obviously, this resistant expression was conditioned by the resistance of their *T. turgidum* and/or *T. tauschii* parents. The overall mean KB infection of the SH was 55.9 % less than the susceptible *T. aestivum* check cultivar 'WL711'.

All the synthetics studied were considered resistant since their reaction fell below the 3 % threshold known to be of practical significance in breeding. Grain with less than 3 % infection can be processed without affecting the quality of the end product. Wheats with more than 3 % infected grains are accepted as grain for livestock feed and priced 20 % less than food wheat (BRENNAN et al. 1990). These genotypes currently represent the best advanced SH wheat lines with good agronomic characteristics, cytogenetic stability and high fertility. They can be readily crossed with common wheat and offer new genetic variability for resistance to KB, that is scarce in *T. aestivum*.

Two strategies are being used for exploiting the germplasm: (i) Exploiting the SH wheats by crosses with susceptible *T. aestivum* cultivars and selecting the resistant segregants, exercising initial caution because of the necrosis genes present in the synthetics as a consequence of the *T. turgidum* cultivars. (ii) From the resistant SH, exploiting the *T. tauschii*

accessions by direct crosses with the elite but susceptible *T. aestivum* cultivars; using recurrent backcrossing with *T. aestivum* parents as the procedure, coupled with cytology to select stable 2n = 6x = 42 euploids (ALONSO and KIMBER 1984, GILL and RAUPP 1987, MUJEEB-KAZI et al. 1993). Studies are underway on the transfer of Karnal bunt resistance genes from SH wheats into bread wheat using the above methods.

Zusammenfassung

Resistenz gegen Karnal-Brand (*Tilletia indica* Mitra) bei synthetischen, aus Kreuzungen zwischen *Triticum turdigum* und *T. tauschii* hervorgegangenen hexaploiden Weizen

Die Resistenz gegen den Karnal-Brand (*Tilletia indica* Mitra) wurde bei synthetischen Hexaploiden (SH) geprüft, die von der CIMMYT unter Einbeziehung von vier Formen von *Triticum turgidum* und neun Formen von *T. tauschii* als Eltern entwickelt worden waren. Die Untersuchungen wurden über drei Vegetationsperioden in drei Jahren in Ciudad Obregon in Mexiko durchgeführt. Zehn zufällig ausgewählte Halme jeder Hexaploiden wurden im Entwicklungsstadium 48—49 nach ZADOKS et al. mit einer wäßrigen Sporensuspension (10 000 Sporen/ml Wasser) injiziert. Zur Reifezeit wurden die inokulierten Ähren einzeln gedroschen und der Prozentgehalt der vom Karnal-Brand (KB) befallenen Körner bestimmt. Gemessen am durchschnittlichen Befall jeder Pflanze in jeder Vegetationsperiode erwiesen sich 49 % der synthetischen Hexaploiden gegenüber dem Karnal-Brand als immun (0 % Infektion). Die synthetischen Hexaploiden zeichneten sich im allgemeinen durch eine hochgradige Resistenz aus, die offensichtlich von ihren Eltern *T. turgidum* und/oder *T. tauschii* herrührte. Gemittelt über alle Werte betrug der Befall der SH-Weizen 0,24 %, während im Vergleich dazu die anfällige Backweizensorte 'WL711' einen Befall von 56,14 % aufwies. Zur Zeit werden in der CIMMYT die KB-Resistenz-Gene von SH-Weizen auf Backweizen übertragen.

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