Karnal bunt resistance in Synthetic Hexaploid wheats (SH) derived from durum wheat x *Aegilops tauschii* combinations and in some SH x bread wheat derivatives

A. Mujeeb-Kazi [1,2,3], G. Fuentes-Davilla [1], Alvina Gul [3] and Javed I. Mirza [2]

1. International Maize and Wheat Improvement Center (CIMMYT), Apartado Postal 6-641, Mexico D. F. 06600, MEXICO
2. National Agricultural Research Center (NARC), Islamabad, PAKISTAN.
3. National Institute for Biotechnology & Genetic Engineering (NIBGE), Faisalabad, PAKISTAN

Summary

Bridge crosses utilizing the D genome synthetic hexaploids (SH), *Triticum turgidum / Aegilops tauschii* (2n = 6x = 42, AABBDD), are a potent means of improving bread wheat (*T. aestivum*) for biotic and abiotic stresses. The synthetic germplasm enables incorporation of the genetic diversity of *T. turgidum* cultivars together with the attributes of the *Ae. tauschii* accessions. In this research, SH wheats were screened for karnal bunt in Obregon, Mexico over six crop cycles and several SHs were earlier identified with an immune response. These SHs have unique *Ae. tauschii* accessions as parents. Phenologicaly descriptors and additional trait evaluations led us to develop a sub-set of the most desirable combinations for wheat breeding. The SH wheats are generally tall, late to mature, have good agronomic type, and are non-free threshing with a high 1000 kernel weight. All have a spring growth habit with several possessing multiple stress resistances. The resistance exhibited by SH wheats has been transferred into elite but KB susceptible bread wheat cultivars thus generating a new and unique genetic resource that can be readily exploited by conventional breeding programs.

Key Words: D genome, interspecific hybridization, karnal bunt resistance, *Aegilops tauschii*

Introduction

There are quarantines against *Neovossia indica* (casual pathogen of the disease karnal bunt) in many countries that limit germplasm exchange and distribution of bread wheat. The fungus was first reported from India (MITRA, 1931). Subsequent reports have also come from Mexico (DURAN, 1972), Pakistan (MUNJAL, 1975) and Nepal (SINGH et al. 1989). The disease affects the grain where dark teliospore masses partially substitute for the endosperm. Fungal teliospores are resistant to physical and chemical treatments and hence making karnal bunt control difficult. Chemical control however, can be accomplished with fungicidal applications during anthesis but they are not economically feasible commercially. Resistant cultivars are the most plausible option for disease control. Though resistance to karnal bunt is

limited in bread wheat, the related progenitor species are a potent source. One strategy and our priority has been to harness the attribute of the diploid D genome progenitor (2n = 2x = 14, DD) through the synthetic hexaploids that are produced from durum wheat x *Aegilops tauschii* hybridizations.
We report here the karnal bunt screening data of an elite synthetic hexaploid set and several other SHs produced later, over six crop cycles of artificial screening in Obregon, Mexico. Also reported is the performance of bread wheat x resistant SH cross derivatives screened in a similar manner over multiple crop cycles.

Materials and Methods

Germplasms

(a) High yielding durum wheat cultivars were hybridized randomly with several *Aegilops tauschii* accessions. The cross products were synthetic hexaploids. From the first 420 SHs produced an elite set of 95 entries was assembled for global usage by wheat researchers. The following 200 were also screened for KB. These SHs were not included in the elite 1 nursery.
(b) Elite high yielding bread wheat cultivars susceptible to karnal bunt were hybridized as maternal or paternal parents to some KB resistant SH wheats. The F1 combinations were advanced by conventional breeding protocols to F_8.

The above germplasms were screened for karnal bunt at the Mexican Institute of Forestry, Agriculture, and Livestock (INIFAP), Campo Agricola Experimental Valle del Yaqui (CAEVY) Research Station in Sonora, Mexico over six crop cycles form 1995 to 2000 by the boot inoculation test (WARHAM, 1984). Susceptible bread wheat checks were WL-711, Flycatcher, Borlaug, Papago, Kauz and Yaco.

KB Screening Protocol

(a) Inoculum preparation

Teliospores from various locations in the Yaqui Valley, Sonora, Mexico were used to ensure a genetically heterogenous composite of the fungus population. To isolate teliospores, infected kernels were shaken in a water-tween-20 solution (2-3 drops of tween-20/100 ml of water) 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 onto 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 enhanced 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 24h using a haemocytometer, and the spore concentration adjusted to 10,000/ml.

(b) Inoculation technique and harvest

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 color-coding tape to indicate the date of inoculation. There were between 2 to 3 dates of planting during each cycle that was tested. At maturity, 10 spikes were graded for infection and the overall percentage infection calculated for each entry.

RESULTS AND DISCUSSION

Germplasm Production

Crosses between elite *T. turgidum* and *Ae. tauschii* accessions resulted in F1 hybrids with 2n = 3x = 21, ABD plants. These hybrid seedlings after colchicine treatment led to hexaploid C-0 (2n=6x=42, AABBDD) seed formation. Stable plants with 42 chromosomes called synthetic hexaploids (SH) were selected; seed was increased and used for biotic stress screening. The germplasm offered abundant diversity for stresses that are significant for global bread wheat cultivation (MUJEEB-KAZI ET AL, 1996).

An elite SH set of 95 entries was prepared from the initial 420 SH wheats. These 95 germplasm lines are maintained and distributed by CIMMYT's germplasm bank. Additional repositories are at the Kansas State University Wheat Genetic Resource Facility, Manhattan, Kansas, USA and at National Agricultural Research Center, Islamabad, Pakistan.

KB Screening of Elite SH lines

High levels of resistance in the elite SH wheats were observed during the KB stress screening. The durum cultivars involved in these SH combinations were generally susceptible under the severe greenhouse inoculation tests. The corresponding SH wheats showed immunity under similar conditions (data not presented). This enabled us to conclude that the SH wheats were resistant because of the respective *Ae. tauschii* accessions involvement. Some of these combinations are listed in Table 1, and represent different *Ae. tauschii* accessions. The susceptible bread wheat cultivar WL711 had a 74.5 % mean infection. These genotypes represent the best SH lines with good agronomic characteristics, cytogenetic stability, and high fertility. The resistant SH germplasm lines identified in the present study are all spring habit types, and can be readily crossed to bread wheat cultivars. Use of SH wheat as a bridge cross is one breeding strategy that allows not only the *Ae. tauschii* resistance diversity to be exploited, but also enables the incorporation of the genetic diversity of the respective durum cultivars. A constraint to be cognizant of is associated with the BW/SH or SH/BW F_1 hybrid necrosis. All SH wheats are non-free threshing, but the simple genetic control readily yields free-threshing segregates (VILLAREAL ET AL. 1996).

Table 1. Some Synthetic hexaploids combinations (*Triticum turgidum / Aegilops taushii*, 2n=6x=42, AABBDD) immune (0%) to karnal bunt following 4 years of artificial screening under field conditions at Ciudad Obregon, Mexico

Pedigree	Cross Number
Doy1/*Ae. tauschii* (188)*	CIGM88.1175-0B
Altar 84/*Ae. tauschii* (193)	CIGM87.2775-1B-0PR-0B
Altar 84/*Ae. tauschii* (198)	CIGM87.2768-1B-0PR-0B
Altar 84/*Ae. tauschii* (205)	CIGM87.2770-1B-0PR-0B
Croc 1/*Ae. tauschii* (213)	CIGM90.412-0B
Altar 84/*Ae. tauschii* (221)	CIGM87.2761-1B-0PR-0B
Croc 1/*Ae. tauschii* (224)	CIGM86.950-1M-1Y-0B-0PR-0B
Yuk / *Ae. tauschii* (217)	CIGM90.561-0B
Altar 84/*Ae. tauschii* (219)	CIGM87.2760-1B-0PR-0B
Altar 84/ *Ae. tauschii* (220)	CIGM87.2760-1B-0PR-0B
68112/Ward//*Ae.tauschii* (369)	CIGM88.1313-0B
WL711 **	Susceptible bread wheat

* *Aegilops tauschii* accession number in CIMMYT's Wheat Wide Crosses working collection

** Mean Karnal bunt infection = 57.7%

Utilization of resistant SH wheats in bread wheat breeding

The immune synthetic hexaploids offer a wide array of D genome genetic diversity for incorporation into KB susceptible but high yielding elite bread wheat cultivars via bridge crossing. The main precaution to be taken is avoiding the use of combinations that give F_1 necrosis. Some of the immune synthetics were crossed several years ago with KB susceptible bread wheat cultivars and their free-threshing advanced derivatives were selected for good agronomic type. These derivatives have been screened under artificial inoculation in the field in Obregon for over five years. The results are reported for thirteen of the best advanced lines (Table 2), where in each case the mean infection level over five years of testing generally remained below 3%. This 3% threshold level is known to be of practical significance in breeding because 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).

FUTURE EXPLOITATION

VILLAREAL ET AL. (1996) registered four SH lines that involved accessions 198, 221, 223 and 224. These SHs were 83 to 103 cm in height with a 1000 kernel weight between 56.7 to 61.3 gm. Subsequently MUJEEB-KAZI ET AL. (2001) registered 10 more SHs with a wider height range (90 to 145 cm) and 1000 grain weight extending

Table 2. Screening for Karnal bunt resistance in some advanced derivatives from Synthetic hexaploid (SH) / bread wheat (BW) combinations across five cycles in Ciudad Obregon, Mexico from 1995-1996 to 1999-2000

Germplasm	Mean percentage infection in each year					
	1995-1996	1996-1997	1997-1998	1998-1999	1999-2000	MEAN
Croc 1 / *Ae. tauschii*(205)** // Fct	1.80	3.70	1.20	0.18	0.47	1.47
Croc 1 / *Ae. tauschii*(205) // Fct	2.60	1.50	2.30	0.08	2.11	1.72
Croc 1 / *Ae. tauschii*(224) // Kauz	0.40	0.00	1.20	0.00	0.00	0.32
Croc 1 / *Ae. tauschii*(224) // Kauz	0.40	0.80	2.10	0.28	0.31	0.78
Croc 1 / *Ae. tauschii*(224) // Kauz	0.10	0.00	4.10	1.81	1.40	1.48
Croc 1 / *Ae.tauschii*(205) // 2* Borl95	0.30	0.01	3.10	0.00	1.50	0.98
Altar 84/ *Ae. tauschii*(221) // Yaco	0.40	1.20	0.71	0.27	2.10	0.94
Croc 1 / *Ae. tauschii*(205) // Kauz	1.77	0.10	1.70	0.00	0.90	0.89
Croc 1 / *Ae. tauschii*(205) // Kauz	1.50	2.20	4.80	1.30	1.10	2.18
Croc 1 / *Ae. tauschii*(205) // Kauz	1.65	0.95	2.89	0.28	1.30	1.41
Croc 1 / *Ae. tauschii*(205) // Borl95	0.00	0.26	2.29	3.31	2.43	1.65
Croc 1 / *Ae. tauschii*(205) // Borl95	0.00	0.00	1.54	0.00	1.90	0.69
Croc 1 / *Ae. tauschii*(213) // Pgo	0.00	3.50	3.49	0.00	3.08	2.01
Flycatcher	39.0	40.1	44.7	39.8	44.4	41.6
Borlaug 95	27.4	27.8	28.8	29.0	28.0	28.2
Papago	16.8	17.9	19.4	16.3	19.1	17.9
Kauz	35.6	35.8	38.9	36.6	40.1	37.4
Yaco	47.0	46.5	49.9	51.4	47.2	48.4
WL711	51.6	50.2	55.8	57.0	54.9	53.9
LSD 0.05	1.94	2.78	3.37	1.85	2.93	

* Infection percentages estimated from artificial field inoculations of 10 spikes per entry.

** *Aegilops tauschii* accession number in CIMMYT's Wheat Wide Crosses working collection at Mexico up to 66.3 gm. Additional accessions were 174, 188, 192, 217, 249, 447, 458, 518 and 629. Several other promising SHs with different accessions were not included in the above reports.

Breeders have utilized the above synthetics for their programs with great interest being given to the increased 1000 kernel weight as a factor for enhancing yield in the cross derivatives. In addition due to extensive screening of the SHs available in CIMMYT, genetic resource flexibility exists that enables us to utilize SHs with good KB resistance coupled with multiple stress resistances. Notable are SHs that possess *Ae. tauschii* accessions 205, 213, 221 and 224 in their pedigrees which have been combined with elite bread wheat cultivars.

With the devolution of selected CIMMYT Wide Cross activities the germplasm mentioned here has been moved to National Agricultural Research Center (NARC), Islamabad, Pakistan where it has been seed increased and is also being evaluated for karnal bunt resistance under Pakistan conditions.

CONCLUSIONS

- Various *T. turgidum x Ae. tauschii* combinations have yielded 620 synthetic hexaploids (SH). From the first batch of 420 SHs produced, an elite set of 95 has been derived for diverse stress tests and international distribution. This set plus the remaining 200 SHs from 421 to 620 were screened for karnal bunt resistance.
- Of the 95 elite SHs only 2 SH combinations had an infection level higher than 5.0% over 3 years of testing across 2 to 3 inoculation dates each year. In the fourth year of testing 53 synthetics had immune (0%) infection across each of the three inoculation dates. Another 28 immune SHs were similarly identified from the remaining 200 SHs tested.
- Some advanced derivatives from immune SH x Susceptible bread wheats tested over 5 years expressed less than 3.0% infection; threshold of practical significance in breeding.

REFERENCES

Brennan, J.P., E.J. Warham, J. Heranadez, D. Byerlee, and F. Coronel. 1990. Economic losses from KB of wheat in Mexico. CIMMYT Economics Working Paper 90/02. CIMMYT, Mexico, D. F., Mexico.

Duran, R., 1972. Further aspects of teliospore germination in North American smut fungi II. **Canadian J. Botany.** 50: 2569-2573.

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

Mujeeb-Kazi, A., V. Rosas and S. Roldan. 1996. Conservation of the genetic variation of *Triticum tauschii* (Coss.) Schmal. (*Aegilops squarrosa* auct. Non L.), in synthetic hexaploid wheats (*T. turgidum* L. s. lat. X *T. tauschii*; 2n=6x=42, AABBDD), and its potential utilization for wheat improvement. **Genetic Resources and Crop Evolution.** 43: 129-134.

Mujeeb-Kazi, A., A. Cortes, R. Delgado, and V. Rosas. 2001. Cytogenetics of bread wheat/*Thinopyrum bessarabicum* derivatives based upon *Ph* and *ph* influence. Agronomy Abstracts. American Society of Agronomy, CD-ROM.

Munjal, R. L. 1975. Status of Karnal bunt *(Neovossia indica*) of wheat in northern India during 1968-69 and 1969-70. Indian J. Mycol. **Plant Pathology.** 5: 185-187.

Singh, D. V., R. Agarwal, J. K. Shrestha, B. R. Thapa, and H. J. Dubin, 1989. First report of Tilletia Indica on wheat in Nepal. **Plant Disease.** 73: 273.

Villareal, R.L., A. Mujeeb-Kazi, and V. Rosas. 1996. Inheritance of threshability in synthetic hexaploid (*Triticum turgidum x T. tauschii*) by *T. aestivum* crosses. **Plant Breeding.** 115: 407-409.

Warham, E.J. 1984. A comparison of inoculation methods for Karnal bunt (*Neovossia indica*). **Phytopathology.** 74: 856-857.

Zadoks, J.C., T.T. Chang, and C.F. Konzak. 1974. A decimal code for the growth stages of cereals. **Weed Research.** 14: 415-421.

Received 5 September, 2005, accepted 3 August, 2006