Bread wheat/D genome synthetic hexaploid derivatives resistant to *Helminthosporium sativum* spot blotch

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ABSTRACT: Spot blotch of wheat caused by *Cochliobolus sativus* Ito et Kuribay (syn. *Helminthosporium sativum*) is one of the major diseases of wheat in tropical areas. Excessive yield losses are not uncommon. Limited conventional primary and perennial tertiary gene pool species have provided resistance diversity for the disease. Additionally, a more diversified and potent source resides in goat grass (*Triticum tauschii,* syn. *Aegilops squarrosa, Ae. tauschii*; 2n=2x=14, DD) accessions. Several *Ae. tauschii* accessions have been combined with *T. turgidum* cultivars to produce 620 synthetic hexaploids (SH). Disease screening in Mexico identified several SHs with superior resistance levels. The resistance of some SH wheats has been transferred into advanced derivatives from bread wheat (BW)/SH crosses. These derivatives have been made homozygous by the sexual haploid methodology, and are designated for global testing. A modified partial monosomic analysis procedure is assisting our efforts to identify the genetic contribution of some of these resistant BW/SH doubled haploids.

INTRODUCTION: Spot blotch (leaf blotch) of wheat (*Triticum aestivum* L.) caused by *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur (syn.: *Bipolaris sorokiniana* (Sacc.) Shoemaker, *Helminthosporium sativum* Pammel, C.M. King & Bakke) is an important pathogen that limits production in many nontraditional hot, humid, wheat producing areas of Asia, Africa, and South America. *Cochliobolus sativus* can attack seedlings, roots, leaves, nodes, spikes, and grains during various stages of plant development. Estimates of yield losses due to spot blotch on wheat vary widely. Because of the importance of this disease, chemical control is applied in order to achieve crop production stability in many parts of the world. Emphasis is also being given to an integrated pest management approach utilizing resistant cultivars, healthy seed, appropriate cultural practices, and chemical sprays. Though breeding for resistance is a high priority, it is hampered by scarcity of adequate resistance within *T. aestivum*.

Sources of resistance to *C. sativus* in species other than *T. aestivum* (i.e., alien gene pools) are of special interest in breeding programs, and we have been making an effort to incorporate and exploit alien resistance genes from the primary gene pool in a wheat background.

Interspecific hybridization that utilizes the genetic diversity of the D genome of *Aegilops tauschii* accessions is our current priority. Elucidated here is the current status of *C. sativus* resistant germplasm that has emanated from use of the *Ae. tauschii* genetic resource.

MATERIALS AND METHODS:

Germplasm development. Elite durum wheat (*Triticum turgidum* L. s. lat.) cultivars were crossed with several hundred *Aegilops squarrosa* accessions. Embryos were rescued, plated in artificial media, differentiated, and yielded F1 hybrids (2n=3x=21, ABD) that upon colchicine treatment produced 2n=6x=42, AABBDD synthetic hexaploids (SH). Accession acquisition and procedures for SH production have been described by Mujeeb-Kazi et al. 1996. These SH wheats are maintained by increasing seed of each combination under controlled conditions by glassine

bagging at least 50 spikes per combination at each increase cycle. Based upon growth performance in two Mexican locations (Ciudad Obregon and El Batan), an elite set of 95 SH entries has been assembled for global distribution.

Screening of the SH germplasm for *C. sativus* resistance. The 620 amphiploid synthetic hexaploid (SH) combinations, their durum parents, the two susceptible 'Ciano 79' and resistant (BH 1146) bread wheat cultivars were each planted in replicated hill plots in Poza Rica, Mexico, for *C. sativus* screening. Disease evaluations were based on foliar infestation and grain blemish at maturity. A double-digit scale measured foliar infestation, where the first digit indicated the height of infection and the second digit, infection severity. Scale gradations were 1 to 9. For height of infection, a score of 5 was for plants with infection up to the plant center, and a score of 9 indicated the infection had spread to the flag leaf. A disease severity score of 1 was for infected leaves exhibiting low disease symptoms, whereas a 9 reflected total leaf destruction. Grain infection at maturity was scored on a 1 to 5 scale, with 1 being low and 5 being high seed blemish at embryo points. Spike appearance was rated as 1 to 9 with 1 being healthy and 9 severely blotched.

Incorporating *C. sativus* resistance from resistant synthetic hexaploids into bread wheat; developing advanced lines and their doubled haploid germplasm. The F_1 hybrids between susceptible bread wheat (BW) cultivars and *C. sativus* resistant synthetic hexaploids (SH) were advanced by conventional breeding protocols. The BW/SH derivatives were screened for *C. sativus* using procedures similar to those described for the SH screening. Highly resistant derivatives formed an elite set of advanced BW/SH lines based upon superior agronomic plant type. Some of these advanced lines were crossed with maize and yielded polyhaploids that upon colchicine treatment gave varied numbers of doubled haploids (DH) and homozygous germplasm.

RESULTS AND DISCUSSION:

Screening synthetic hexaploid wheats for *C. sativus*. From our screening of the 620 synthetic wheats, 45 SH *C. sativus* resistant entries were selected. The durum cultivars involved in these SH combinations were susceptible to *C. sativus* with leaf scores of 9-7 to 9-9, grain infection between 3 to 5, and spike damage score between 7 to 9. Since the selected SH wheats were superior, their resistance was interpreted to be a consequence of the respective *Ae. tauschii* accessions involvement. The SH entries identified for wheat improvement had leaf scores of 9-2 to 9-3, seed damage of 2 to 3, and a disease infection score on spikes at maturity of 1 or 2.

Production and screening of BW/SH advanced lines. The SH bridge is advantageous for wheat improvement, since it allows not only the *Ae. tauschii* resistance to be exploited but also incorporates the genetic diversity of the A and B genomes of the respective durum wheat cultivars. Using this approach we have developed bread wheat/diverse SH germplasms from which several lines resistant to *C. sativus* have been identified. These BW/SH lines express similar resistances as their SH parents and have been further selected for desirable plant type, maturity, and rust resistances. The best of these agronomically superior lines were made homozygous using the sexual DH methodology (Mujeeb-Kazi and Riera-Lizarazu, 1996). The haploid procedure allows for frequencies of about 20% embryo excision, 80% plantlet regeneration, and 70% colchicine induced doubling. These DH *C. sativus* resistant germplasms are being registered and will be distributed for international testing in several locations.

Modified partial genetic analysis. Considering that the durum cultivars are susceptible to *C. sativus,* the resistance observed in the SH wheats is inferred to be a contribution of the *Ae. tauschii* D genome. Five resistant BW/SH derivatives are now undergoing a modified genetic analysis procedure. The homozygous DH derivatives are crossed onto the 1D to 7D monosomes; 41 chromosome monosomic plants from each F_1 are cytologically identified and used for polyhaploid (n=3x=21) plus double haploid (2n=6x=42) production. Non-segregating, resistant 1D to 7D double haploids are expected to possess the *Ae. tauschii* accession gene(s) contributing to resistance.

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