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AEGILOPS TAUSCHII, AS A SPOT BLOTCH (*COCHLIOBOLUS SATIVUS*) RESISTANCE SOURCE FOR BREAD WHEAT IMPROVEMENT

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Abstract

Cochliobolus sativus the cause of Spot blotch, affects wheat crops across several environments globally with Bangladesh being represented as a major disease location. Accessional diversity in conventional germplasm was limited until the mid-eightees with BH-1146 being most popular. With the novel usage of exotic germplasm emerging in that decade, wide cross programs got popularity and the release of the intergeneric cross product “Mayoor” initiated additional efforts around this new genetic diversity resource of the wheat family Triticeae. Derivatives of Mayoor known as Chirya added another dimension and then on the scene came the D genome contribution of the diploid progenitor *Aegilops tauschii* (2n=2x=14). Synthetic hexaploid wheats (SH) derived from crosses between *Triticum turgidum* x *Ae. tauschii* (2n=6x=42; AABBDD) became a popular germplasm source for tests against various wheat production constraints around biotic and abiotic stresses and these provided superior resistance to *C. sativus* either alone or also in SH x SH combinations due to gene pyramiding. From the earlier resistance scores of 9-5 *vs* 9-9 for susceptibility the SH germplasm was from 9-2 or 9-3 to pyramided lines with 2-2 or 3-2. The latter translated to the same 2-2 or 3-2 levels when these superior SH’s were crossed onto elite bread wheat cultivars susceptible to *C. sativus.* Further, the resistant stocks derived from both the intergeneric and interspecific avenues gave seed finish readings of 1 or 2 *vs* the 4 or 5 susceptible germplasm scores. This paper elucidates data of the above three categories of *C. sativus* tests done under stringent field conditions over several years of evaluations.

Introduction

Cochliobolus sativus the cause of Spot blotch, affects wheat across several environments from Latin America, Africa, Asia and Southeast Asia with Bangladesh being represented as a major disease location. The pathogen is capable of attacking seedlings, roots, leaves, nodes, spikes and grains during the various crop developmental stages. Yield loss estimates are variable ranging from low damage to the upper limit of upto 85.0% (Raemakers, 1988). Due to the importance of the disease across several wheat production environments several control measures are in adoption. The recognized potent control measure is *via* a genetic route. This route is however handicapped by scarcity of desirable genetic diversity. Novel options are thus adopted where exploiting the close and distant bread wheat relatives as a source of new alleles to provide stability are progressively demonstrating significant promise (Mujeeb-Kazi, 2003). Utilizing these novel resources involves intergeneric and interspecific hybridization strategies. The former approach around *Thinopyrum curvifolium* has been most promising, leading to registration of genetic stocks (Mujeeb-Kazi *et al.*, 1995) and a varietal release

"AzubiCiat" in Bolivia (Villareal *et al*., 1995). The interspecific avenue has gauged the utility of various *Aegilops tauschii* accessions for countering the pathogens virulent effects. This diploid grass (2n=2x=14, DD) has shown enormous promise in providing extensive diversity for resistances and tolerances to various biotic and abiotic stress constraints that limit wheat production globally (Mujeeb-Kazi *et al*., 2007) and is another highly prioritized diversity resource being exploited by wheat breeders. Initial results of this grasses accessional contributions were reported by Mujeeb-Kazi *et al*., (1996). In this paper we report the performance of some primary *Ae. tauschii* genetic stocks commonly known as 'synthetic hexaploids or SH wheats' and their advanced breeding lines derived from combining these resistant stocks with high yielding but *C. sativus* susceptible bread wheat cultivars based upon their performance at a severe screening site in Mexico at Poza Rica (lat 21 N and 60 m elevation).

Materials and Methods

Germplasms

a. Synthetic hexaploids (2n=6x=42, AABBDD) derived from crosses between elite durum wheat (*Triticum turgidum*, 2n=4x=28, AABB) cultivars and several accessions of *Aegilops tauschii* (2n=2x=14, DD) syn. *Ae. squarrosa* or *T. tauschii* were the novel germplasm resource to be screened for *Cochliobolus sativus*.

b. Resistant SH wheats with *Ae. tauschii* accessional diversity based upon DNA fingerprinting were hybridized to give F_1 combinations, then advanced to F_2 from which the superior resistant derivatives were selected and made homozygous by the wheat / maize crossing procedure (Mujeeb-Kazi, 2000). The doubled haploids (DH's) were seed increased and screened for *C. sativus* resistance.

c. Advanced lines from high yielding but *C. sativus* susceptible bread wheat cultivars crossed with resistant SH germplasm and their progeny (BW/SH) advanced for stability intermingled with *C. sativus* screening.

Disease screening: The above germplasms were planted in the field at Poza Rica (20/32N, 97/26W; 60 m altitude) and screened for *C. sativus* (Spot blotch) over five growing cycles during the months of November and March. The location is a natural severe epidemic site. SH wheats were planted in replicated hill plots and the BW / SH derivatives in 2m replicated double rows. Ciano 79 and Mayoor were the susceptible and resistant bread wheat cultivars.

Disease evaluations were based upon foliar infection and grain blemish at maturity (Saari & Prescott 1975). A double digit scale measured foliar infection where the first digit equated to the height of infection and the second digit with infection severity. The scale gradations were 1 to 9. For the height of infection a score of 5 was for plants with infection upto the plant center and for a score of 9 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 score reflected total leaf destruction. Grain infection at maturity was scored on a 1 to 5 scale with 1 being low and 5 being a high seed blemish at its embryo points.

Results and Discussion

a. Screening of the synthetic hexaploid germplasm: Within the main wide crossing program at CIMMYT several synthetic hexaploid wheats have been produced by randomly crossing elite durum wheat cultivars with the globally available *Ae. tauschii* accessions generating 1014 synthetics (Mujeeb-Kazi, 2003). Screening of these synthetic wheats has enabled sub-sets to be assembled for various biotic and abiotic stresses thus allowing targeted utilization by global wheat breeders for their preferred wheat production local stress constraints. The *C. sativus* screening done on the main SH group allowed the grouping of several excellent SH's that not only were ideal for *C. sativus* resistance but also possessed other beneficial phenological attributes. Data gathered over five test cycles summarized as mean values (Table 1) indicate the superiority of these synthetics over the susceptible wheat control "Ciano 79" with equivalent similarity or superiority over the resistant check "Mayoor". All SH's had the double digit scores between 9-2 and 9-3 as compared to the 9-9 for Ciano and 9-3 for Mayoor. Grain finish scores were between 1 and 2 vs 5 for Ciano and 2 for Mayoor. Significant differences prevailed amongst the SHs for days to flowering, maturity, height and grain finish that have positive value for use in wheat breeding. The SH's in general are tall with a range from 100 to110 cms but are still suitable as resistant genetic stocks since appropriate height segregates can be readily selected from breeding progenies. Several synthetics had grain finish scores of 1 upon harvest. This score reflects that the grains are well filled and have no blemish. In each of these synthetics the pedigree shows the involvement of a different *Ae. tauschii* accession and thus inferred as an apparent manifest of unique diversity. Future molecular diagnostics can unravel the DNA polymorphism profile of each synthetic; information that when combined with other phenological traits like 1000 kernel weight, pubescence and leaf waxiness would permit targeted use of a SH entry in wheat breeding. The field conditions in Poza Rica over the last 2 to 3 weeks prior to maturity are hot and humid and day temperatures approaching 40°C are not uncommon. Hence with a seed finish score of 1 is an encouragement to test such SH entries for heat tolerance in other sites permitting another important abiotic stress to be addressed. Useful additional diversity in these synthetics is also present in the A and B genomes of the durum parent involved in the pedigree. A significant contribution of these genomes would be towards the 1000 kernel weight that is of immediate value for elevating yield per se.

b. Screening of doubled haploids from SH X SH F_2 combinations around gene pyramiding: The SH's involved represent diverse *Ae. tauschii* accessional gene pyramiding efforts and were developed by intercrossing several *T. turgidum* / *Ae. tauschii* combinations involving different *Ae. tauschii* accessions. From segregating F_2 populations, superior spot-blotch individual resistant plants were selected and hybridized with *Zea mays*. The resulting haploids (n=3x=21, ABD) were colchicine treated to yield homozygous doubled derivatives (2n=6x=42, AABBDD) with high disease resistance levels. Figure 1 elucidates the schematic of the development protocol. Some of the best doubled haploid lines are listed in Table 2. The superior performance of these lines is indicative of stress attributes pyramiding reflected by disease scores of 3-2 or lower and grain finish appearance after harvest with ratings of less than 2 versus the susceptible scores of 9-9 and 4, respectively. Use of these lines in breeding provides enormous advantage as the combination of alleles provide a distinct advantage in transferring multiple *Ae. tauschii* accessional traits simultaneously promoting output swiftness towards applied alien gene transfer programs. This strategy opens up doors for adopting a research course for combining other SHs for other resistant traits. This strategic exercise will prove important since F2 based superior resistant SH's derived *via* doubled haploidy would place various potent genes together. To advance such a program on a large scale, assessment of the diversity in desired resistant SHs using molecular markers would be necessary for which D genome specific SSR markers would be preferred. Hence a combination of stress screening, molecular diversity and conventional gene pyramiding mediated by induced homozygosity would be an efficient integrated investigative course to ensure durability of resistance to wheat.

c. Screening of advanced derivatives from bread wheat (BW)/SH crosses: *C. sativus* resistant SH's were crossed onto high yielding but *C. sativus* susceptible bread wheats (BW). The F_1 combinations were advanced by the pedigree system and subjected to field screening pressures of the disease in Poza Rica that allowed resistant segregants to be identified (Fig. 1). Utilization of SH's around their gene pyramiding structure has yielded advanced BW / SH derivatives that are all in the score categories of 1-1, 2-2 to 3-2 over a progressive disease scoring system from 60 to 96 days. Their seed finish scores range between 1 to 2 and all have desired characters of ideal height, days to physiological maturity and 1000 kernel weight. All the above attributes are essential for wheat improvement and demonstrate how a resistant SH genetic stock can be put to use in a breeding program.

In earlier work on rust resistance, researchers had systematically screened *Ae. tauschii* accessions and identified some with resistance. When synthetics were produced using these accessions the resulting SH wheats did not manifest the trait creating an usage impediment for practical use associated with genomic based suppression (Ma *et al*., 1995 and cited references within). Thus having identified *C. sativus* resistance in SHs (Table1 and 2) provided us with a starting donor point from which the desired transfer was needed into elite but *C. sativus* susceptible wheat cultivars. Would this transfer be expressed in the wheat X SH crossed derivatives or would suppression occur ?. Data of Table 3 elucidates that the derivatives in all cases carry the resistance trait emanating from *Ae. tauschii* derived synthetic germplasm across various contributing accessions. The derivatives are all reservoirs of superior resistance as indicated by the slow disease progress curve, low disease coefficient of infection score at near maturity, and excellent grain finish values compared to the susceptible control and the resistant check Mayoor. The agronomic traits of days to flowering, days to maturity and height at maturity of all derivatives observed reflect a desirable wheat plant type. The late maturity and taller plant height of the synthetics was favorably modified (Table 3) and the germplasm made more user friendly for international evaluation for *C. sativus* screening. The most promising line derived from these BW / SH combinations was Mayoor//TKSN10181/*Ae. tauschii* (222) that also possessed resistance to various other stresses like *Fusriaum graminearum* Type 1 to 4, *Septoria tritici*, *Neovossia indica* and all three rusts (leaf, stem and stripe). A mapping population has been developed by crossing this line with a bread wheat cultivar that is susceptible to all the above ten biotic stresses and has DNA fingerprint diversity (Fig. 2). This population will not only serve to address QTL mapping areas for *C. sativus* but also aid in targeting the other stresses around the standard phenotyping / genotyping procedures. The agronomic performance of the above BW X SH *C. sativus* resistant line is superior. The line has a slow disease progress curve, adequate height, normal maturity and good grain finish and appearance (Fig. 3 a to c). Its performance is distinctly superior (Fig. 3a) to the susceptible check 'Ciano 79' (Fig. 3b, left) and many notches better than the resistant elite cultivar 'Chirya' or 'AzubiCiat' (Fig. 3b, right).

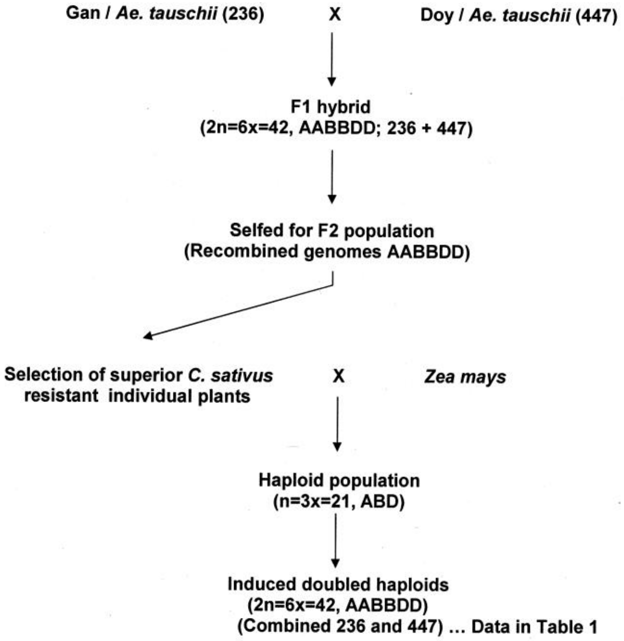

Fig. 1. Schematic elucidating the pyramiding of resistance from two *Cochliobolus sativus* resistant synthetics possessing diverse *Aegilops tauschii* accessions.

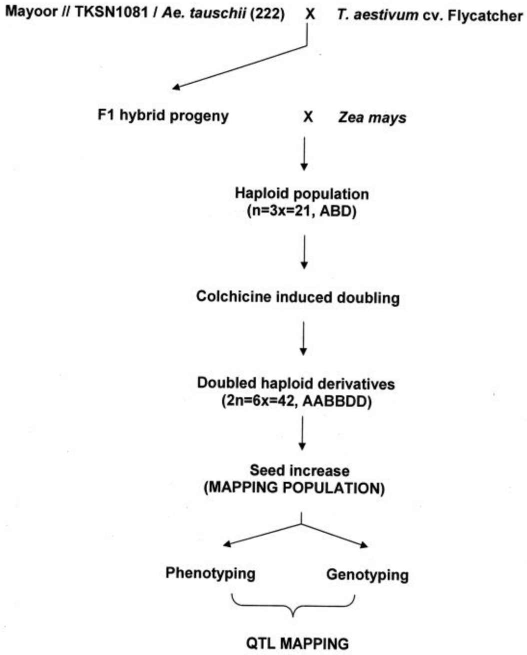

Fig. 2. Development schematic of the *Cochliobolus sativus* resistant advanced line based molecular mapping population with the susceptible wheat cultivar ‘Flycatcher’ targeted for QTL mapping.

Fig. 3. Performance of the advanced bread wheat / synthetic hexaploid derivative against *Cochliobolus sativus* under field conditions showing in A): the advanced line with superior resistance and score of 1-1 which ended at maturity at 2-2, in B left) the susceptible check ‘Ciano 79” scored 9-9, in B right) the resistant check AzubiCiat or Chirya at 2-1 and ending at maturity at 9-3 and in C) seed finish of 1-1 for the BW/SH advanced line.

Introduction of the germplasm and studies in Pakistan: A major wheat improvement effort in Pakistan has relied upon acquisition of germplasm from foreign programs (predominantly from CIMMYT, Mexico) followed by its cultivation, observing their performance for prioritized biotic/abiotic stresses, selecting those best adapted, entering these into national yield trials and releasing new varieties. In brief this introduction, adaptation and varietal release strategy has dominated Pakistan's wheat cultivation. On a lesser level, well structured/resourceful breeding programs do have a recombination breeding program that have also significantly contributed to the national wheat cultivation scenario. In both these categories of wheat improvement the diversity exploited is from the primary gene pool, is conventional and easy to introgress around established field protocols of breeding within any program. There is a risk in such programs that repeated use of top-class varieties in computational/permutational crossings may lead to an eventually narrow germplasm base generating vulnerability to pathogens. Triticeae species belonging to its three gene pools (Jiang *et al*., 1995) offer unique diversity that has not been aggressively utilized in breeding programs and provides an allelic resource of enormous potential. Wheat cultivation in Pakistan has seen the huge contributions of only one major alien Triticeae species i.e., *Secale cereale* through its spontaneous Robertsonian translocation event giving the T1BL.1RS wheats, of which Pak-81, Pirsabak 85 etc., form key known Pakistani wheat examples. Global focus of an applied nature utilizing alien species was actively initiated in CIMMYT, Mexico some three decades ago and from that program *via* introduction, adaptation and varietal release emerged two cultivars for irrigated and rainfed environments named Pasban 90 and Rohtas 90 with the former still recognized as a varietal standard for salinity tolerance. The alien source was *Thinopyrum distichum* (2n=4x=28).. Like *S. cereale* this donor also belonged to the tertiary gene pool from which transfers are known to be complex and time consuming essentially due to lack of chromosomal homoeology (Mujeeb-Kazi, 2006). With the advent of molecular scenarios DNA polymorphisms got in demand and the species providing the solution was the D genome diploid progenitor *Ae. tauschii* for molecular map building. Simultaneously this resource provided an opportunity for its numerous accessions to be exploited for wheat improvement. Hence about two decades ago emerged a "rebirth of synthetics" for this applied facet (Mujeeb-Kazi *et al*., 2007). The taxing task of assembling the *Ae. tauschii* diversity across most of its 750 accessions was completed in CIMMYT, Mexico during 1987 and 1998 and just like any other international seed nursery the entire set of SH wheats were introduced into Pakistan. One major difference however was present. These synthetics served as raw materials for stress screening and potential resistant / tolerant sources could be identified. In order to harness these positive observations for wheat improvement, recombination breeding was essential which was targeted around this *C. sativus* set of materials even though *C. sativus* was not a major wheat production constraint in Pakistan the earlier observed linkage of the trait with heat tolerance was of our local interest. From the germplasms introduction we have increased the seed amounts that is an arduous task for the hard to thresh synthetics. The free-threshing germplasm has been evaluated for its agronomic adaptation and the seed increase has provided material for testing in the near future for heat tolerance; a stress that has drawn increased breeding focus due to late wheat plantings across the country because of the cotton / wheat and rice / wheat cropping system practice that makes wheat vulnerable to heat stress at the end of the growth cycle. The molecular mapping population of 169 doubled haploids has also been seed increased and would be ideal germplasm to phenotype for heat tolerance and the various rusts thereby unravelling the genetic contribution coming from the wild relative diploid donor resource.

Conclusions

Unique genetic diversity emanating from the diploid D genome donor *Ae. tauschii* (2n=2x=14, DD) has been accessed and incorporated into synthetic hexaploid wheats (2n=6x=42, AABBDD) by crossing the numerous accessions of the grass with elite but *C. sativus* susceptible durum cultivars (2n=4x=28, AABB). These synthetics upon screening have provided various combinations that are resistant to the blotch stress. Due to durum susceptibility, resistance in the SH germplasm is unequivocally attributed to the *Ae. tauschii* accessions. Interline SH combinations have extended the utility of the individual resistant SHs by pyramiding their different alleles mediated by wheat/maize doubled haploidy involvements. From this first step of having diverse resistant genetic stocks available for use in breeding, the next step was to transfer resistance to bread wheats and obtain desirable agronomic plant type segregates. This has been satisfactorily achieved and the BW/SH derivatives express superior resistance to *C. sativus* across the main evaluation parameters. The germplasm output has also paved the way to develop molecular mapping populations opening up avenues for conducting inheritance and mapping studies. The primary synthetics have become a rich resource for studying molecular diversity targeted around the D genome contributions from various *Ae. tauschii* accessions. On the applied front of wheat production in Pakistan this germplasm has the potency for being assessed for heat tolerance as the resistant selections for *C. sativus* were made under hot humid conditions in a very severe location that may prove beneficial for our national productivity constraints around this abiotic parameter that is of growing concern.

References

Jiang, J., B. Friebe and B.S. Gill. 1994. Recent advances in alien gene transfer in wheat. *Euphytica*, 73: 199-212.

Mujeeb-Kazi, A. 2000. An analysis of the use of haploidy in wheat improvement. In: *Application of biotechnologies to wheat breeding.* (Eds.): M.M. Kohli and F.M. Francis. La Estanzuela, Uruguay. Nov. 19-20, 1998. p. 33-48.

Mujeeb-Kazi, A. 2003. New genetic stocks for durum and bread wheat improvement. *Tenth International Wheat Genetics Symposium, Paestum, Italy*. pp. 772-774.

Mujeeb-Kazi, A. 2006. Utilization of genetic resources for bread wheat Improvement. CRC Series, (Eds.): R.J. Singh and P.P. Jauhar. pp. 61-97.

Mujeeb-Kazi, A., A. Gul, M. Farooq, S. Rizwan and I. Ahmad. 2007. Rebirth of synthetic hexaploids and global implications for wheat improvement. *Australian Journal of Agricultural Research* (IN PRESS).

Mujeeb-Kazi, A., R.L. Villareal, L.A. Gilchrist and S. Rajaram. 1996. Registration of five wheat germplasm lines resistant to *Helminthosporium* leaf blight. *Crop Science*, 36: 216-217.

Mujeeb-Kazi, A., V. Rosas and S. Roldan. 1996. Conservation of the genetic variation of *Triticum tauschii* (Coss.) Schmalh. (*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.

Raemakers, R. 1988. *Helminthosporium sativum*: Disease complex on wheat and sources of resistance in Zambia. In: *Wheat production constraints in tropical environments,* (Ed.): A.R. Klatt, CIMMYT, Mexico, D.F. pp. 175-186.

Saari, E.E. and J.M. Prescott. 1975. A scale of appraising the foliar intensity of wheat diseases. *Plant Disease Rep*., 59: 377-380.

Villareal, R.L., A. Mujeeb-Kazi, L.I. Gilchrist and E. Del-Toro. 1995. Yield loss to spot blotch in spring bread wheat in warm non-traditional wheat production areas. *Plant Disease*, 79: 893-897.

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