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Identification of Intergeneric and Synthetic Spring Wheat Lines with Resistance to *Fusarium* Head Blight.

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Key Words: intergeneric wheat hybrids, synthetic wheat, *Agropyron repens*, *Fusarium graminearum*, disease resistance, pollen fertility

Abstract

Fusarium head blight (FHB) is a serious disease in many cereal crops worldwide, as it can drastically reduce both yield and quality in infected crops. Resistance to FHB is therefore a desirable trait for incorporation into new wheat cultivars. Wild relatives are a source of new genetic variation and have been shown to enhance disease resistance when hybridized with wheat. This research has focused on evaluating intergeneric and interspecific wheat lines for resistance to FHB. Intergeneric hybrids of wheat (*Triticum aestivum*) and *Agropyron repens* syn. *Elytrigia repens*, and synthetic wheat lines produced at CIMMYT, were evaluated under greenhouse conditions for resistance to FHB caused by *Fusarium graminearum*. Approximately 35% of the intergeneric lines and 15% of the synthetic lines consistently displayed moderate to high levels of Type II resistance. Pollen staining was conducted to determine the fertility levels of the intergeneric lines. 79% of the F₃ lines and 72% of the F₅ and F₆ lines displayed fertility levels above 75%. Control cultivars displayed fertility levels ranging from 88-94%. Generally, lines that rated as moderately to highly resistant were also highly fertile. These lines could be of significant value in wheat breeding programs aimed at integrating new sources of resistance to fusarium head blight.

Introduction

Fusarium head blight (FHB), principally caused by *Fusarium graminearum*, is a serious disease in wheat and other cereal crops around the world as it reduces both yield and quality. Mycotoxins produced by *F. graminearum*, primarily deoxynivalenol (DON), make infected grains unsuitable for human or livestock consumption. Milling and baking quality is also compromised by starch and protein degradation in tombstone kernels (Clear and Patrick, 2000; Ruckebauer et al., 2001). The development of resistant varieties is the most economical and effective method of managing this disease. Resistance to fusarium head blight has been identified in several hexaploid wheat sources around the world, and attempts to introduce this resistance into adapted varieties are underway. Sumai 3, a Chinese cultivar, has been one of the most widely used sources of FHB resistance to date (Wang et al., 2001). The improvement of FHB resistance is limited by the narrow genetic variability in wheat (Chen et al., 2001), so it is important to search for alternative sources of resistance. Wild relatives are a source of a large amount of genetic variation, and they have many desirable traits, such as disease resistance, that can be introgressed into the wheat genome. Wide hybridization of alien species with wheat is a valuable technique for increasing the amount of genetic diversity in wheat, allowing for greater

advancements in producing FHB resistant varieties. The objective of this research is to evaluate and identify novel sources of Type II resistance in intergeneric and synthetic wheat lines. Because *F. graminearum* infects mainly through extruded anthers and many of the intergeneric lines display signs of sterility, the fertility of these lines was also determined to ensure against false resistance ratings.

Materials and Methods

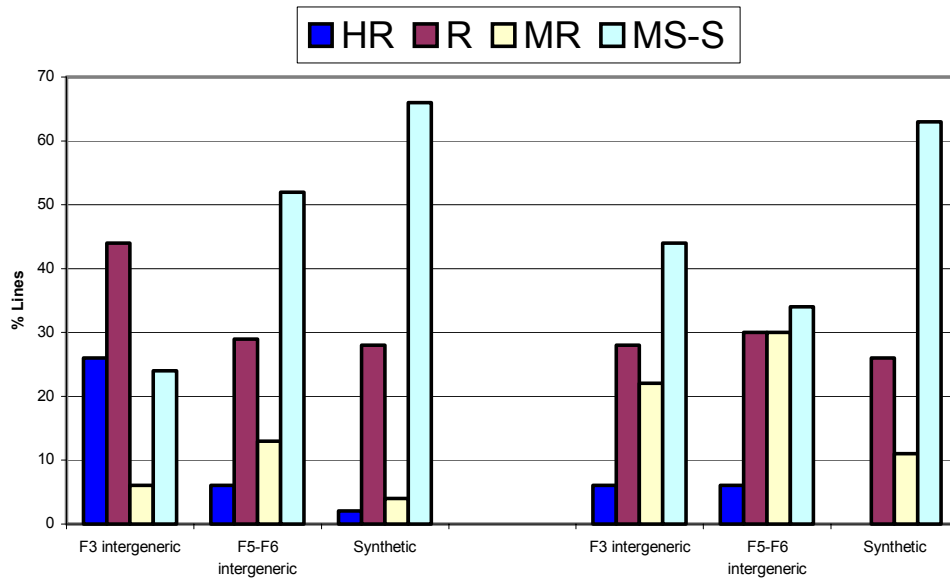
Intergeneric hybrids have been generated from wheat cv. Crocus (Zale and Scoles, 1999) x *Agropyron repens* syn. *Elytrigia repens*, and backcrossed to several spring wheat cultivars. These BC₁F₂-derived lines were evaluated for resistance to FHB as two separate groups of lines advanced to the F₃ (34 lines) and F₅-F₆ (78 lines) generations, respectively. The third set of material, consisting of 47 synthetic spring wheat lines identified as being possible sources of FHB resistance, was obtained from CIMMYT. Lines were replicated and arranged in a completely randomized design in a greenhouse along with check varieties Sumai 3 (resistant to highly resistant), Alsen (moderately resistant), AC Barrie (moderately resistant), and CDC Teal (susceptible). Two point inoculation techniques were used to screen for FHB resistance in these lines: the canaryseed and the spore suspension methods. In the canaryseed point inoculation method, kernels of canaryseed colonized by *F. graminearum* were placed between the lemma and the palea of one floret of a central spikelet at early anthesis using a pair of fine forceps. For the spore suspension method, the concentration of *F. graminearum* spores was adjusted to 10000 spores per mL of suspension. A pipettor was used to inject 10 μ L of suspension into one floret of a central spikelet at early anthesis. The canaryseed method was used during the initial screening of the two groups of intergeneric material, while the spore suspension method was utilized for the both screenings of the synthetic wheat lines and the second screening of the intergeneric lines. Inoculated plants were placed in a mist chamber for 24 hours, then rated 21 days following inoculation using a 0-5 rating scale adapted from Griffey et al. (1999). The average of the spike ratings at 21 days for each line was used to evaluate the degree of resistance to FHB. Lines that were identified as moderately resistant to highly resistant in the first disease screening experiment were tested a second time using the spore suspension method.

All of the intergeneric lines were grown in the field during the summer of 2003 to determine their fertility levels. Check cultivars used for this experiment were Sumai 3, Alsen, AC Barrie, and CDC Teal. At early anthesis, two heads from each line were collected and fixed in Carnoy's solution for 24 hours, then transferred to 70% alcohol. Anthers from the primary floret of a central spikelet were extracted and stained in IKI. Stained vs. unstained pollen grains were counted to determine the fertility levels of each line.

Results

In the first set of intergeneric material, 76% of the lines rated as MR-HR in the first screening (Figure 1). 26% were highly resistant, 44% were resistant, and 6% were moderately resistant. Only 24% of the lines were moderately susceptible to susceptible. The mean FHB rating was 2.7, indicating that this group of lines, on average, was resistant to FHB. When the MR-HR lines were evaluated a second time, only 56% of those were MR-HR, and 44% were MS-S, with 6%

HR, 28% R, and 22% MR. The mean rating of these lines was MR. After both screenings, 29%



of the F₃ intergeneric lines were identified as containing moderate to high resistance to FHB.

*HR= highly resistant, R= resistant, MR= moderately resistant, MS-S= moderately susceptible to susceptible

Figure 1. Percentage of lines from three sets of material displaying various levels of resistance to FHB.

The second group of wheat x quackgrass lines was screened twice using the canaryseed inoculation method before the MR-HR lines were selected for spore suspension point inoculation testing. Hot, humid greenhouse conditions during the summer of 2002 provided an environment conducive to a high degree of pathogen infection, and the resistant checks displayed a high level of infection. To minimize the influence of high outdoor temperatures on disease ratings, screening of this material was repeated during the winter and averaged for all of the lines. 6% of the lines were highly resistant, 29% were resistant and 13% displayed moderate resistance (Figure 1). Susceptible lines made up 52% of this material. The mean FHB rating for this material was 3.3, which indicates this group of lines is moderately resistant to FHB. Over both screenings, 39% of these lines showed moderate to high resistance to FHB.

The ratings data for the synthetic spring wheat lines indicated that 2% of the lines were highly resistant to FHB, while 28% were resistant and 4% were moderately resistant (Figure 1). The mean FHB rating for this set of lines was 3.5, which indicates moderate resistance to moderate susceptibility to FHB. The second screening of the moderately to highly resistant lines identified only 15% of the entire set of synthetic wheat lines as being potential sources of resistance.

In the pollen fertility experiment, the check varieties ranged from fertility levels of 88-94%. The F₃ intergeneric lines had a mean fertility of 80%±12%, with 79% of the material over 75% fertile (Figure 2), while the F₅-F₆ lines had a mean fertility 76%±10% with 72% of the lines over 75% fertile (Figure 3). These two groups of material were not significantly different in pollen fertility. When fertility levels were compared to resistance ratings (Table 1), sterility was not a major issue in the moderately to highly resistant lines. Table 1 shows a sample of the most resistant lines from the intergeneric populations and their fertility levels. The highlighted line indicates that its fertility level is quite low, and it could be a sterile line.

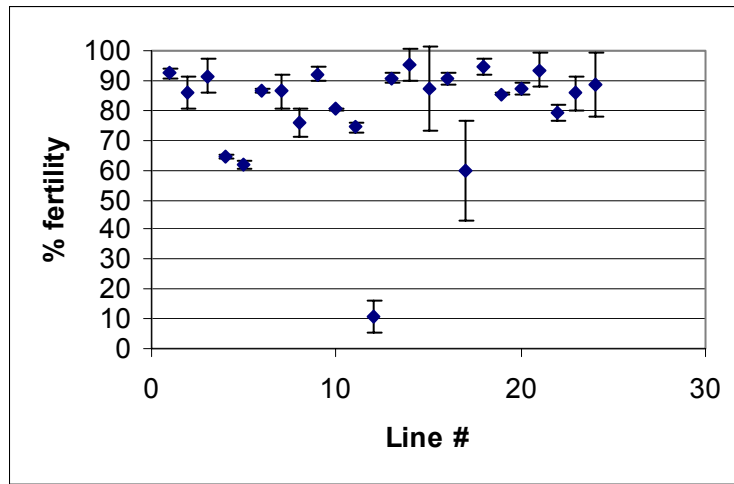


Figure 2. Fertility of F₃ intergeneric lines grown in the field.

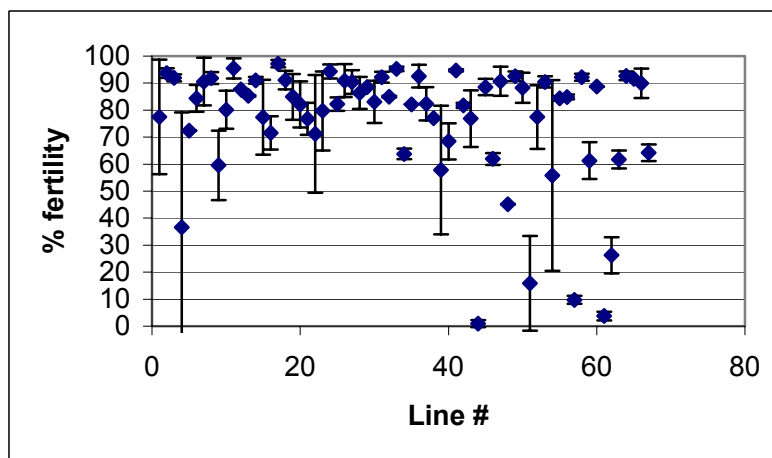


Figure 3. Fertility of F₅-F₆ intergeneric lines grown in the field.

Table 1. Fertility levels of resistant and highly resistant intergeneric lines

F3 Line	% Fertility	F5-F6 Line	% Fertility
00SF-A26-1	93%	00Ar-2-3	37%
00SF-A26-2	86%	00Ar-5-3	92%
00SF-A50-2	81%	00Ar-47-2	91%
00SF-A62-2	91%	00Ar-50-1	78%
00SF-A85-1	88%	00Ar-54-1	85%
00SF-A85-2	94%	00Ar-57-1	76%
Croc/Ar-1	86%	00Ar-57-2	72%

Discussion

The intergeneric wheat x *A. repens* lines in the F₅-F₆ generation had the highest percentage of lines that exhibited moderate to high resistance to FHB (Figure 1), while the synthetic lines were the least resistant. Additional susceptible lines were identified during the second screening of all material due to differences in outdoor conditions at different times of the year that affected greenhouse temperatures, humidity levels, and disease production. The F₃ intergeneric population could still be segregating for resistance to FHB, which could also have contributed to differences in the ratings over both screenings. Sterility was generally not a problem in the intergeneric lines.

Future considerations

Cytogenetic characterization of the most resistant lines will be carried out to evaluate the degree of intergeneric introgression in the wheat x *A. repens* populations. The best lines in terms of FHB resistance and fertility will be backcrossed to various bread wheat lines to transfer the resistance into an adapted wheat background and remove undesirable alien characteristics. The identification of several lines containing moderate to high resistance in all three populations suggests that alien material could be a valuable source for enhancing resistance to fusarium head blight in spring wheat breeding programs.

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