DETECTION OF SEQUENCES CONFERRING RESISTANCE TO CEPHALOSPORIUM STRIPE DISEASE IN WHEAT VIA IN SITU HYBRIDIZATION WITH A THINOPYRUM-SPECIFIC TRANSPONSON PROBE

ABSTRACT
The fungal pathogen Cephalosporium gramineum causes strip disease in cereal crops, including wheat and can cause a loss of over 50 percent in yield due to decreased seed size and reduced seed production. We investigated Cephalosporium-resistant wheat lines developed at Washington State University. The pedigrees of these wheat lines incorporate parents from the wheatgrass Thinopyrum, which has an ability to withstand a variety of crop diseases including Cephalosporium stripe disease. We hypothesize that sequences retained from the Thinopyrum lineage are responsible for the disease resistance in these lines. We incorporated into the wheat genome through chromosomal translocation or DNA crossover. We isolated a Thinopyrum-specific transposon sequence from wheat-Thinopyrum amphiploids. This transposon was fluorescently labeled and employed as a probe for FISH in situ hybridization to detect regions of Thinopyrum-determined disease resistance in the resistant wheat lines WA7970, WA7971 and WA8000 as well as in control lines. Our use of a genome-specific transposon probe is intended to provide greater binding specificity than our previous methods employing fluorescently-labeled whole genomic DNA, which can bind to common sites on both Thinopyrum and wheat chromosomes.

BACKGROUND
The fungal pathogen Cephalosporium gramineum attacks wheat and other cereal crops like rye and winter barley. Wheat is the major economic host for this fungal disease, which can cause a loss of over 50 percent in yield due to decreased seed size and reduced seed production. First reported in North America in 1955, Cephalosporium stripe disease has been found in all Midwestern and Northwestern states. Currently, many crop varieties lack a resistance to this fungus.

Researchers from the Winter Wheat Breeding lab at Washington State University developed wheat lines that incorporate wheatgrass parents from the genus Thinopyrum. Thinopyrum wheatgrass has an ability to withstand a variety of crop diseases including Cephalosporium stripe disease. These developed wheat lines also have resistance against C. gramineum (Table 1). It is hypothesized that the Thinopyrum genes for disease resistance were incorporated into the wheat genome through transposon chromosomal translocation or DNA crossover.

To investigate our hypothesis, we conducted fluorescent genomic in situ hybridization (FISH) on metaphase chromosomes from three advanced breeding lines that have Thinopyrum parents in their lineage.

Methods

Table 1. Cephalosporium stripe disease index and % seed infected by Cephalosporium gramineum for four winter wheat varieties grown at the Palouse Conservation Field Station, Pullman, 2006.

<table>
<thead>
<tr>
<th>Variety/Site</th>
<th>% infected seed</th>
<th>Disease index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elton</td>
<td>0.01</td>
<td>43.7</td>
</tr>
<tr>
<td>Madsen</td>
<td>0.10</td>
<td>47.8</td>
</tr>
<tr>
<td>Amphiploid</td>
<td>0.00</td>
<td>37.7</td>
</tr>
</tbody>
</table>


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CYTOLOGY RESULTS

WA7970

WA7971

WA8000

PERENNIAL HYBRID CONTROL

REFERENCES

FUNDING
This work was made possible through the Washburn University Transformational Experience Scholarly/Creative grant fund.

CONCLUSIONS & FUTURE DIRECTIONS

• Our initial FISH results failed to detect any “hotspots” of probe-binding, despite strong binding to controls. Non-specific hybridization was markedly increased over controls, possibly due to a lack of high-association binding sites.

• Thinopyrum-specific transposon pleUCD2 was chosen as an alternate probe. Binding efficiency of a ple2 probe should be increased and purified using the SuperGlo (Invitrogen) kit. Following transposon isolation, ~100% of the isolated transposon sequence was repeatable after gel extraction, as described above, but substituting the DNEF solution with one containing a mixture of 0.5 M KHP and 0.1 M HCl-6K9F.

METHODS

Chromosome Prep & in situ Hybridization

A 66 cm long wheat tester line was pre-grown for 24 hours at 64°C, then for 48 hours in 0.2% p-chloroanisole and squashed under a coverslip. Slides were frozen at -80°C (then dehydrated at 40% acetic acid and 95% ethanol and chromosomal DNA was denatured by heating in 70% formamide at 70°C for 2 minutes. Genomic DNA probe was prepared using the BioNick nick translation system (Invitrogen). A transposon DNA sample was prepared by autoclaving Chinese Spring genomic DNA till it sheared to length of 200-300 nt. A high hybridization solution of 2% deionized formaldehyde, 60% formamide, 0.2X SSC, 50 ng of biotinylated probe and 2.5 μg of blocking DNA was added to each slide and incubated for 36 hours at 37°C. Excess probe was removed through a rinse in 2X SSC. Slides were oscillated in a solution of avidin-fluorescein, followed by biotinylated-anti-avidin and a second incubation in avidin-fluorescein. Slides were washed thrice with 0.025 M acetic acid, 0.15 M sodium chloride.

DNA Extraction & Transposon Isolation

Amphiploid DNA was isolated as described above, but substituting the dNTP solution with one containing 0.1M dATP and 0.1M biotin-14-dATP. The PCR solution consisted of 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.5 mM MgCl2, 200 μM dNTP, 1.0 U Taq polymerase (NEB) and 10 μM oligonucleotide primer.

PCR of the isolated transposon was amplified using the following primers:

pLe2 TRANSPONSON ISOLATION

PCR of wheat/wheatgrass amphiploids and chromosome addition lines. The transposon probe was fluorescently labeled and employed as a probe for FISH in situ hybridization.

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