(54) Title: GENETIC LOCI ASSOCIATED WITH RESISTANCE TO TROPICAL RUST IN MAIZE

(57) Abstract: The invention relates to methods and compositions for identifying maize plants that have increased or decreased resistance to tropical rust. The methods use molecular markers to identify and select plants with increased resistance to tropical rust or to identify and counter-select plants with decreased resistance to tropical rust. Maize plants generated by the methods of the invention are also a feature of the invention. Also of interest are methods used to correlate allelic variation with a trait of interest.
wo 2011/056836 A: — without international search report and to be republished upon receipt of that report (Rule 48.2(g)) — with sequence listing part of description (Rule 5.2(a))
CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 61/257,977, filed November 4, 2009, which is incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present disclosure relates to compositions and methods useful in enhancing resistance to tropical rust in plants and methods to identify allelic variations associated with a trait of interest.

BACKGROUND OF THE INVENTION


Disease management strategies include crop rotation, destruction of old maize residues by tillage, and fungicide application, all of which are aimed at reducing the fungal inoculum. However, the most effective and most preferred method of control for tropical rust is the planting of resistant hybrids.

The methods of controlling tropical rust by reducing fungal inoculum require additional time and resources on the part of the farmer, and in addition, can have detrimental effects on the environment. This makes the planting of resistant hybrids even more attractive to farmers and the general public. Thus, it is desirable to provide compositions and methods for identifying and selecting maize plants with enhanced resistance to tropical rust.

SUMMARY OF THE INVENTION

Compositions and methods for identifying and selecting maize plants with enhanced resistance to tropical rust are provided. Also provided are methods for marker assisted selection of plants that have enhanced resistance to tropical rust.
In one embodiment, methods for selecting maize plants or germplasm with enhanced resistance to tropical rust by detecting the presence of at least one allele of a first marker locus that is linked to and associated with the "T" deletion at position 16 of PHMTR (SEQ ID NO:155) or the "GAG" haplotype at positions 337-339 of reference sequence SEQ ID NO:167 and selecting the maize plants or germplasm that comprise the at least one allele of a first marker locus that is linked to and associated with the "T" deletion at position 16 of PHMTR (SEQ ID NO:155) or the "GAG" haplotype at positions 337-339 of reference sequence SEQ ID NO:167 are provided. The at least one allele of the first marker locus can be linked to and associated with the "T" deletion at position 16 of PHMTR (SEQ ID NO:155) or the "GAG" haplotype at positions 337-339 of reference sequence SEQ ID NO:167 by up to 20 cM on a single meiosis map.

In another embodiment, methods for selecting maize plants or germplasm with enhanced resistance to tropical rust by detecting the "T" deletion at position 16 of PHMTR (SEQ ID NO:155) or the "GAG" haplotype at positions 337-339 of reference sequence SEQ ID NO:167; and selecting the maize plants or germplasm that comprise the "T" deletion at position 16 of PHMTR (SEQ ID NO:155) or the "GAG" haplotype at positions 337-339 of reference sequence SEQ ID NO:167 are provided.

In another embodiment, methods for identifying maize plants with enhanced resistance to tropical rust by detecting a marker locus in the genome of the maize plant using the sequence of the marker locus, a portion of the sequence of the marker locus, or a complement of the sequence of the marker locus, or of a portion thereof, as a marker probe, are provided. The marker probe hybridizes under stringent conditions to the contiguous DNA between and including SEQ ID NO:89, or a nucleotide sequence that is 95% identical to SEQ ID NO:89 based on the Clustal V method of alignment, and SEQ ID NO:96, or a nucleotide sequence that is 95% identical to SEQ ID NO:96 based on the Clustal V method of alignment, and the marker locus comprises at least one allele that is associated with the enhanced resistance to tropical rust.

In another embodiment, methods for identifying maize plants with enhanced resistance to tropical rust by detecting at least one marker allele associated with the enhanced resistance in the germplasm of the maize plant are provided. The marker
locus can be selected from any of the following marker loci: PHM1 192-26-U, PHM1 192-4-U, C00435-802-U, C00436-801-U, PHM187-7-U, C00423-801-U, PHM5028-24-U, PHM13818-15-U, PHM15721-39-U, PHM15721-180-U, C00441-801-U, C00441-802-U, PHM4370-19-U, PHM731-107-U, C00071-01-U, PHM8249-21-U, C00428-801-U, PHM18427-13-U, PHM9535-10-U, PHM9535-6-U, PHM9535-7-U, and PHM4003-1 3-U; the PHM markers PHM15590, PHM13818, PHM1 192, PHM187, PHM5028, PHM4370, PHM731, and PHM15721; Sub2e, Sub9d, Sub19c, Sub23m, C06621-1-K2, and C06621-1-K4; as well as any other marker that is linked to these markers. The marker locus can also be found within any of the following intervals on chromosome 10 comprising and flanked by:

i. PHM15590 and PHM9535;
ii. PHM15590 and PHM15721;
iii. C00441 and C00428;
iv. PHM731 and PHM15721; and
v. C00071 and PHM731.

The marker locus comprises at least one allele that is associated with enhanced resistance to tropical rust.

In another embodiment, methods for identifying maize plants with enhanced resistance to tropical rust by detecting a haplotype in the germplasm of the maize plant that is associated with enhanced resistance to tropical rust are provided. The haplotype comprises alleles at one or more marker loci, wherein the one or more marker loci are found within any of the following intervals on chromosome 10 comprising and flanked by:

i. PHM15590 and PHM9535;
ii. PHM15590 and PHM15721;
iii. C00441 and C00428;
iv. PHM731 and PHM15721; and
v. C00071 and PHM731.

The haplotype can comprise a "T" deletion at position 16 of PHMTR or "GAG" at positions 337-339 of reference sequence SEQ ID NO:167.

In another embodiment, methods of selecting maize plants with enhanced resistance to tropical rust are provided. In this method, a first maize plant is obtained wherein the maize plant has at least one allele of a marker locus that is
located within any of the following intervals on chromosome 10 comprising and flanked by:

i. PHM15590 and PHM9535;
ii. PHM15590 and PHM15721;
iii. C00441 and C00428;
iv. PHM731 and PHM15721; and
v. C00071 and PHM731;

and the allele is associated with enhanced resistance to tropical rust. The first maize plant is crossed to a second maize plant, and the resulting progeny plants are evaluated for the allele of the first maize plant. Progeny plants that possess the allele of the first maize plant are then selected as having enhanced resistance to tropical rust.

In another embodiment, methods of selecting maize plants with enhanced resistance to tropical rust are provided. In this method, a first maize plant is obtained wherein the maize plant comprises in its genome the "T" deletion at position 16 of PHMTR or the "GAG" haplotype at positions 337-339 of reference sequence SEQ ID NO:167. The first maize plant is crossed to a second maize plant, and the resulting progeny plants are evaluated for the "T" deletion at position 16 of PHMTR or the "GAG" haplotype at positions 337-339 of reference sequence SEQ ID NO:167. Progeny plants that possess the "T" deletion at position 16 of PHMTR or the "GAG" haplotype at positions 337-339 of reference sequence SEQ ID NO:167 are then selected as having enhanced resistance to tropical rust.

Additionally, maize plants identified or selected by the methods described above, wherein the plant is not CML339, are of interest. Furthermore, progeny of maize plants identified or selected by the methods described above are of interest.

In another embodiment, methods of identifying allelic variations associated with a desirable form of a trait are presented. In these methods, raw sequences are aligned with an open:extension cost ratio greater than 10 and background noise is removed by trimming the tails. Random allelic variation is then trimmed, and an unweighted pair group method with arithmetic mean (UPGMA) is applied. The trimming of random allelic variation and the application of UPGMA to the alignment are repeated until a phenogram is identified. Allelic variations associated with the phenotype of interest can then be identified from the phenogram.
BRIEF DESCRIPTION OF FIGURES AND SEQUENCE LISTINGS

The invention can be more fully understood from the following detailed description and the accompanying drawings and Sequence Listing which form a part of this application. The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in Nucleic Acids Research 13:3021-3030 (1985) and in the Biochemical Journal 219 (No. 2): 345-373 (1984), which are herein incorporated by reference in their entirety. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. § 1.822.

FIG. 1 shows the physical map arrangement of sequenced BACs (obtained from the Maize Genome Browser, which is publicly available on the internet) that assemble to the chromosome 10 region defined by and including BACs c0497L12 and b0191 E02. The positions of the PHM markers described herein (region defined by and including PHM15590 and PHM15721) are indicated, as are the positions of the public markers lying within the interval.

FIGs. 2A and 2B show the frequency distributions of PH468 x PHS6Y F2 population for tropical and southern rust scores, respectively.

FIG. 3 shows the composite interval mapping results obtained using the PH468 x PHS6Y F2 population. A peak of significance was identified on the short arm of chromosome 10. Marker positions on the x-axis correspond to the PHB genetic map. The y-axis represents the LOD score.

FIG. 4 (a) Susceptible inbred line and corresponding resistant conversion using PHS6Y as donor parent. (b) Hybrid made with a susceptible version of an inbred. (c) Hybrid made with the resistant ("converted") version of the same inbred. This shows that the tropical rust gene has a dominant effect in the hybrid level.

FIG. 5 shows a hybrid that is highly susceptible to tropical rust (on left) and the same hybrid that has been converted to have enhanced resistance from PHS6Y (on right).

FIG. 6 shows the public BAC clones used as reference for primer design to genotype maize lines that are resistant and susceptible to tropical rust. Internal information regarding BAC overlap was used to further narrow the sequence order of the 2-2.5 Mb region into 24 sub-regions.
FIG. 7 shows part of the reference sequence (top) obtained by the genotyping of maize lines resistant and susceptible to tropical rust using PCR primers (SEQ ID NOs: 133 and 134) designed for clone ID C9050c064G1 1c (Table 9). SEQ ID NOs: 137-142 represent amplicons obtained from resistant lines, while SEQ ID NOs: 143-154 represent amplicons obtained from susceptible lines. The area highlighted in grey represents a 21 bp-region of the reference sequence (referred to as PHMTR; SEQ ID NO: 155). Maize lines having a T-deletion at bp 16 (indicated by the arrow) all showed enhanced resistance to tropical rust and have the sequence of SEQ ID NO: 156.

FIG. 8 shows part of the alignment of amplicon sequences obtained using primers SEQ ID NO: 135 and SEQ ID NO: 136. A "GAG" haplotype (boxed) was found to be unique to all lines with enhanced resistance to tropical rust.

The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. § 1.821-1.825. The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in Nucleic Acids Res. 13:3021-3030 (1985) and in the Biochemical J. 219 (2):345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. § 1.822.

Table 1 lists the sequences described herein that are associated with the InvaderPlus Production markers, along with the corresponding identifiers (SEQ ID NO:) as used in the attached Sequence Listing.

<table>
<thead>
<tr>
<th>Marker Name</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Sense</th>
<th>Allele 1</th>
<th>Allele 2</th>
<th>Dye 1</th>
<th>Dye 2</th>
<th>Probe 1</th>
<th>Probe 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHM1 192-26-U</td>
<td>1</td>
<td>2</td>
<td>ANTI-SENSE</td>
<td>G</td>
<td>A</td>
<td>FAM</td>
<td>RED</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>PHM1 192-4-U</td>
<td>5</td>
<td>6</td>
<td>SENSE</td>
<td>D</td>
<td>I</td>
<td>FAM</td>
<td>RED</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>C00435-802-U</td>
<td>9</td>
<td>10</td>
<td>SENSE</td>
<td>A</td>
<td>T</td>
<td>RED</td>
<td>FAM</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>C00436-801-U</td>
<td>13</td>
<td>14</td>
<td>SENSE</td>
<td>A</td>
<td>G</td>
<td>RED</td>
<td>FAM</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>PHM187-7-U</td>
<td>17</td>
<td>18</td>
<td>ANTI-SENSE</td>
<td>G</td>
<td>A</td>
<td>FAM</td>
<td>RED</td>
<td>19</td>
<td>20</td>
</tr>
</tbody>
</table>
Table 2 lists the sequences described herein that are associated with the PHM markers, along with the corresponding identifiers (SEQ ID NO:) as used in the attached Sequence Listing.

<table>
<thead>
<tr>
<th>Marker Locus</th>
<th>Amplicon reference sequence (SEQ ID NO:)</th>
<th>Primer</th>
<th>Forward Primer (SEQ ID NO:)</th>
<th>Reverse Primer (SEQ ID NO:)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHM15590</td>
<td>89</td>
<td>Internal</td>
<td>98</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>External</td>
<td>97</td>
<td>100</td>
</tr>
<tr>
<td>PHM13818</td>
<td>90</td>
<td>Internal</td>
<td>102</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td></td>
<td>External</td>
<td>101</td>
<td>104</td>
</tr>
<tr>
<td>PHM1192</td>
<td>91</td>
<td>Internal</td>
<td>106</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td></td>
<td>External</td>
<td>105</td>
<td>108</td>
</tr>
<tr>
<td>TYPE</td>
<td>numbered</td>
<td>Primers</td>
<td>Ct values</td>
<td>DNA sequence</td>
</tr>
<tr>
<td>--------</td>
<td>----------</td>
<td>------------------</td>
<td>-----------</td>
<td>--------------</td>
</tr>
<tr>
<td>PHM187</td>
<td>92</td>
<td>Internal</td>
<td>110</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td></td>
<td>External</td>
<td>109</td>
<td>112</td>
</tr>
<tr>
<td>PHM5028</td>
<td>93</td>
<td>Internal</td>
<td>114</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td></td>
<td>External</td>
<td>113</td>
<td>116</td>
</tr>
<tr>
<td>PHM4370</td>
<td>94</td>
<td>Internal</td>
<td>118</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td></td>
<td>External</td>
<td>117</td>
<td>120</td>
</tr>
<tr>
<td>PHM731</td>
<td>95</td>
<td>Internal</td>
<td>122</td>
<td>123</td>
</tr>
<tr>
<td></td>
<td></td>
<td>External</td>
<td>121</td>
<td>124</td>
</tr>
<tr>
<td>PHM15721</td>
<td>96</td>
<td>Internal</td>
<td>126</td>
<td>127</td>
</tr>
<tr>
<td></td>
<td></td>
<td>External</td>
<td>125</td>
<td>128</td>
</tr>
</tbody>
</table>

SEQ ID NO:129 is the L primer designed for Clone ID Ct9050c497L12e.
SEQ ID NO:130 is the R primer designed for Clone ID Ct9050c497L12e.
SEQ ID NO:131 is the L primer designed for Clone ID Ct9050c064G1 1d.
SEQ ID NO:132 is the R primer designed for Clone ID Ct9050c064G1 1d.
SEQ ID NO:133 is the L primer designed for Clone ID Ct9050c064G1 1c.
SEQ ID NO:134 is the R primer designed for Clone ID Ct9050c064G1 1c.
SEQ ID NO:135 is the L primer designed for Clone ID Ct9050b191 E02m.
SEQ ID NO:136 is the R primer designed for Clone ID Ct9050b191 E02m.
SEQ ID NO:137 is the sequence of the amplicon obtained using SEQ ID NO:133 and SEQ ID NO:134 as the primers and PHS6Y DNA.
SEQ ID NO:138 is the sequence of the amplicon obtained using SEQ ID NO:133 and SEQ ID NO:134 as the primers and PH1JG22 DNA. PH1JG22 is a maize inbred line that is resistant to tropical rust.
SEQ ID NO:139 is the sequence of the amplicon obtained using SEQ ID NO:133 and SEQ ID NO:135 as the primers and PH1 FT71 DNA. PH1 FT71 is a maize inbred line that is resistant to tropical rust.
SEQ ID NO:140 is the sequence of the amplicon obtained using SEQ ID NO:133 and SEQ ID NO:135 as the primers and PH1G3H1 DNA. PH1G3H1 is a maize inbred line that is resistant to tropical rust.
SEQ ID NO:141 is the sequence of the amplicon obtained using SEQ ID NO:133 and SEQ ID NO:135 as the primers and PH1JG01 DNA. PH1JG01 is a maize inbred line that is resistant to tropical rust.
SEQ ID NO:142 is the sequence of the amplicon obtained using SEQ ID NO:133 and SEQ ID NO:135 as the primers and PHP7W DNA. PHP7W is a maize inbred line that is resistant to tropical rust.

SEQ ID NO:143 is the sequence of the amplicon obtained using SEQ ID NO:133 and SEQ ID NO:135 as the primers and PH7W3 DNA. PH7W3 is a maize inbred line that is susceptible to tropical rust.

SEQ ID NO:144 is the sequence of the amplicon obtained using SEQ ID NO:133 and SEQ ID NO:135 as the primers and PH9VF DNA. PH9VF is a maize inbred line that is susceptible to tropical rust.

SEQ ID NO:145 is the sequence of the amplicon obtained using SEQ ID NO:133 and SEQ ID NO:135 as the primers and PHBNA DNA. PHBNA is a maize inbred line that is susceptible to tropical rust.

SEQ ID NO:146 is the sequence of the amplicon obtained using SEQ ID NO:133 and SEQ ID NO:135 as the primers and PH2JR DNA. PH2JR is a maize inbred line that is susceptible to tropical rust.

SEQ ID NO:147 is the sequence of the amplicon obtained using SEQ ID NO:133 and SEQ ID NO:135 as the primers and PHOTJ DNA. PHOTJ is a maize inbred line that is susceptible to tropical rust.

SEQ ID NO:148 is the sequence of the amplicon obtained using SEQ ID NO:133 and SEQ ID NO:135 as the primers and PH467 DNA. PH467 is a maize inbred line that is susceptible to tropical rust.

SEQ ID NO:149 is the sequence of the amplicon obtained using SEQ ID NO:133 and SEQ ID NO:135 as the primers and PH48F DNA. PH48F is a maize inbred line that is susceptible to tropical rust.

SEQ ID NO:150 is the sequence of the amplicon obtained using SEQ ID NO:133 and SEQ ID NO:135 as the primers and PH7WC DNA. PH7WC is a maize inbred line that is susceptible to tropical rust.

SEQ ID NO:151 is the sequence of the amplicon obtained using SEQ ID NO:133 and SEQ ID NO:135 as the primers and 625 DNA. 625 is a maize inbred line that is susceptible to tropical rust.

SEQ ID NO:152 is the sequence of the amplicon obtained using SEQ ID NO:133 and SEQ ID NO:135 as the primers and PHP3P1 DNA. PHP3P1 is a maize inbred line that is susceptible to tropical rust.
SEQ ID NO:1 53 is the sequence of the amplicon obtained using SEQ ID NO:1 33 and SEQ ID NO:1 35 as the primers and PHY7M2 DNA. PHY7M2 is a maize inbred line that is susceptible to tropical rust.

SEQ ID NO:1 54 is the sequence of the amplicon obtained using SEQ ID NO:1 33 and SEQ ID NO:1 35 as the primers and PH147G5 DNA. PH147G5 is a maize inbred line that is susceptible to tropical rust.

SEQ ID NO:1 55 is the sequence of the PHMTR region.

SEQ ID NO:1 56 is the sequence of the PHMTR region without the "T" at position 16 of SEQ ID NO:1 55.

SEQ ID NOs:157-164 are the sequences for primers C06621-1-K2 and C06621-1-K4 (Table 3).

Table 3: C06621-1-K2 and C06621-1-K4 KASP Marker Information

<table>
<thead>
<tr>
<th>Marker Name</th>
<th>C06621-1-K2</th>
<th>C06621-1-K4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverse Primer for Marker 1 (Target Specific)</td>
<td>SEQ ID NO:1 57</td>
<td>SEQ ID NO:1 61</td>
</tr>
<tr>
<td>Reverse Primer for Marker 2 (Internal control)</td>
<td>SEQ ID NO:1 58</td>
<td>SEQ ID NO:1 62</td>
</tr>
<tr>
<td>Allele1</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Allele2</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Dye1</td>
<td>VIC</td>
<td>VIC</td>
</tr>
<tr>
<td>Dye2</td>
<td>FAM</td>
<td>FAM</td>
</tr>
<tr>
<td>Forward Primer for Marker 1 + VIC universal Sequence (Target Specific)</td>
<td>SEQ ID NO:1 59</td>
<td>SEQ ID NO:1 63</td>
</tr>
<tr>
<td>Forward Primer for Marker 2 + FAM universal Sequence (Internal Control)</td>
<td>SEQ ID NO:1 60</td>
<td>SEQ ID NO:1 64</td>
</tr>
</tbody>
</table>

SEQ ID NO:1 65 is the FAM universal sequence.

SEQ ID NO:1 66 is the VIC universal sequence.

SEQ ID NO:1 67 is the reference sequence for Sub23M.
DETAILED DESCRIPTION

The present invention provides allelic compositions in maize and methods for identifying and for selecting maize plants with enhanced resistance to tropical rust. Also within the scope of this invention are allelic compositions and methods used to identify and to counter-select maize plants that have decreased resistance to tropical rust. The following definitions are provided as an aid to understand this invention.

The term "Enhanced resistance", "increased resistance " or "newly conferred resistance" are used interchangeable and refers to an increased level of resistance against a particular pathogen, a wide spectrum of pathogens, or an infection caused by the pathogen(s). An increased level of resistance against a particular fungal pathogen, such tropical rust, for example, constitutes "enhanced" or improved fungal resistance. The embodiments of the invention will enhance or improve fungal plant pathogen resistance, such that the resistance of the plant to a fungal pathogen or pathogens will increase, which in turn, will increase resistance to the disease caused by the fungal pathogen. The term "enhance" refers to improve, increase, amplify, multiply, elevate, raise, and the like. Herein, plants of the invention are described as having "enhanced resistance" to tropical rust infection, as a result of specific alleles at the locus of the invention.

A maize plant that displays enhanced resistance to tropical rust is a plant that is less affected with respect to yield and/or survivability or other relevant agronomic measures, upon introduction of the causative agents of that disease. Resistance is a relative term, indicating that the infected plant produces better yield of maize than another, similarly treated, more susceptible plant. That is, the conditions cause a reduced decrease in maize survival and/or yield in a resistant maize plant, as compared to a susceptible maize plant. One of skill will appreciate that maize plant resistance to tropical rust varies widely, can represent a spectrum of more resistant or less resistant phenotypes, and can vary depending on the severity of the infection. However, by simple observation, one of skill can determine the relative resistance or susceptibility of different plants, plant lines or plant families to tropical rust, and furthermore, will also recognize the phenotypic gradations of "resistant". As used in the art, "resistance" is sometimes referred to as "general resistance", "rate-reducing resistance", or "partial resistance".
"Disease resistance" is a characteristic of a plant, wherein the plant avoids the disease symptoms that are the outcome of plant-pathogen interactions, such as maize-tropical rust interactions. That is, pathogens are prevented from causing plant diseases and the associated disease symptoms, or alternatively, the disease symptoms caused by the pathogen are minimized or lessened. One of skill in the art will appreciate that the compositions and methods disclosed herein can be used with other compositions and methods available in the art for protecting plants from pathogen attack.

As used herein, "fungal resistance" refers to enhanced resistance or tolerance to a fungal pathogen when compared to that of a wild type plant. Effects may vary from a slight increase in tolerance to the effects of the fungal pathogen (e.g., partial inhibition) to total resistance such that the plant is unaffected by the presence of the fungal pathogen.

A plant referred to herein as "diploid" has two sets of chromosomes.

A plant referred to herein as a "doubled haploid" is developed by doubling the haploid set of chromosomes. A doubled haploid plant is considered a homozygous plant.

An "elite line" is any line that has resulted from breeding and selection for superior agronomic performance.

The term "allele" refers to one of two or more different nucleotide sequences that occur at a specific locus.

"Allele frequency" refers to the frequency (proportion or percentage) of an allele within a population, or a population of lines. One can estimate the allele frequency within a population by averaging the allele frequencies of a sample of individuals from that population.

An "amplicon" is an amplified nucleic acid, e.g., a nucleic acid that is produced by amplifying a template nucleic acid by any available amplification method (e.g., PCR, LCR, transcription, or the like).

The term "amplifying" in the context of nucleic acid amplification is any process whereby additional copies of a selected nucleic acid (or a transcribed form thereof) are produced. Typical amplification methods include various polymerase based replication methods, including the polymerase chain reaction (PCR), ligase mediated methods such as the ligase chain reaction (LCR) and RNA polymerase
Based amplification (e.g., by transcription) methods.

The term "assemble" applies to BACs and their propensities for coming together to form contiguous stretches of DNA. A BAC "assembles" to a contig based on sequence alignment, if the BAC is sequenced, or via the alignment of its BAC fingerprint to the fingerprints of other BACs. Public assemblies can be found using the Maize Genome Browser, which is publicly available on the internet.

An allele is "associated with" a trait when it is part of or linked to a DNA sequence or allele that affects the expression of a trait, and the presence of the allele is an indicator that the desired trait or trait form will occur in a plant comprising the allele.

A "BAC", or bacterial artificial chromosome, is a cloning vector derived from the naturally occurring F factor of Escherichia coli. BACs can accept large inserts of DNA sequence. In maize, a number of BACs, or bacterial artificial chromosomes, each containing a large insert of maize genomic DNA, have been assembled into contigs (overlapping contiguous genetic fragments, or 'contiguous DNA').

"Backcrossing" refers to the process whereby hybrid progeny are repeatedly crossed back to one of the parents. The "donor" parent refers to the parental plant with the desired gene/genes, locus/loci, or specific phenotype to be introgressed. The "recipient" parent (used one or more times) or "recurrent" parent (used two or more times) refers to the parental plant into which the gene or locus is being introgressed. For example, see Ragot, M. et al. (1995) Marker-assisted backcrossing: a practical example, in Techniques et Utilisations des Marqueurs Moleculaires Les Colloques, Vol. 72, pp. 45-56, and Openshaw et al., (1994) Marker-assisted Selection in Backcross Breeding, Analysis of Molecular Marker Data, pp. 41-43. The initial cross gives rise to the F1 generation; the term "BC1" then refers to the second use of the recurrent parent, "BC2" refers to the third use of the recurrent parent, and so on.

A centimorgan ("cM") is a unit of measure of recombination frequency. One cM is equal to a 1% chance that a marker at one genetic locus will be separated from a marker at a second locus due to crossing over in a single generation.

As used herein, the term "chromosomal interval" designates a contiguous linear span of genomic DNA that resides in planta on a single chromosome. The genetic elements or genes located on a single chromosomal interval are physically
linked. The size of a chromosomal interval is not particularly limited. In some aspects, the genetic elements located within a single chromosomal interval are genetically linked, typically with a genetic recombination distance of, for example, less than or equal to 20 cM, or alternatively, less than or equal to 10 cM. That is, two genetic elements within a single chromosomal interval undergo recombination at a frequency of less than or equal to 20% or 10%.

As used herein, the term "chromosomal interval" designates a contiguous linear span of genomic DNA that resides in planta on a single chromosome. The genetic elements or genes located on a single chromosomal interval are physically linked. The size of a chromosomal interval is not particularly limited. In some aspects, the genetic elements located within a single chromosomal interval are genetically linked, typically with a genetic recombination distance of, for example, less than or equal to 20 cM, or alternatively, less than or equal to 10 cM. That is, two genetic elements within a single chromosomal interval undergo recombination at a frequency of less than or equal to 20% or 10%.

A "chromosome" is a single piece of coiled DNA containing many genes that act and move as a unit during cell division and therefore can be said to be linked. Can also be referred to as a "linkage group".

The phrase "closely linked", in the present application, means that recombination between two linked loci occurs with a frequency of equal to or less than about 10% (i.e., are separated on a genetic map by not more than 10 cM). Put another way, the closely linked loci co-segregate at least 90% of the time. Marker loci are especially useful in the present invention when they demonstrate a significant probability of co-segregation (linkage) with a desired trait (e.g., pathogenic resistance). Closely linked loci such as a marker locus and a second locus can display an inter-locus recombination frequency of 10% or less, preferably about 9% or less, still more preferably about 8% or less, yet more preferably about 7% or less, still more preferably about 6% or less, yet more preferably about 5% or less, still more preferably about 4% or less, yet more preferably about 3% or less, and still more preferably about 2% or less. In highly preferred embodiments, the relevant loci display a recombination a frequency of about 1% or less, e.g., about 0.75% or less, more preferably about 0.5% or less, or yet more preferably about 0.25% or less. Two loci that are localized to the same chromosome, and at such a
distance that recombination between the two loci occurs at a frequency of less than 10% (e.g., about 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.75%, 0.5%, 0.25%, or less) are also said to be "proximal to" each other. In some cases, two different markers can have the same genetic map coordinates. In that case, the two markers are in such close proximity to each other that recombination occurs between them with such low frequency that it is undetectable.

In bioinformatics, "clustering" refers to the grouping of sequences that are somehow related and is often used to make a non-redundant set of representative sequences. The sequences can be genomic, "transcriptomic" (ESTs) or protein in nature.

The term "complement" refers to a nucleotide sequence that is complementary to a given nucleotide sequence, i.e. the sequences are related by the base-pairing rules.

The term "contiguous DNA" refers to an uninterrupted stretch of genomic DNA represented by partially overlapping pieces or contigs.

When referring to the relationship between two genetic elements, such as a genetic element contributing to resistance and a proximal marker, "coupling" phase linkage indicates the state where the "favorable" allele at the resistance locus is physically associated on the same chromosome strand as the "favorable" allele of the respective linked marker locus. In coupling phase, both favorable alleles are inherited together by progeny that inherit that chromosome strand.

The term "crossed" or "cross" means the fusion of gametes via pollination to produce progeny (e.g., cells, seeds or plants). The term encompasses both sexual crosses (the pollination of one plant by another) and selfing (self-pollination, e.g., when the pollen and ovule are from the same plant). The term "crossing" refers to the act of fusing gametes via pollination to produce progeny.

A plant referred to herein as "diploid" has two sets (genomes) of chromosomes.

A plant referred to herein as a "doubled haploid" is developed by doubling the haploid set of chromosomes (i.e., half the normal number of chromosomes). A doubled haploid plant has two identical sets of chromosomes, and all loci are considered homozygous.
An "elite line" is any line that has resulted from breeding and selection for superior agronomic performance.

An "exotic maize strain" or an "exotic maize germplasm" is a strain or germplasm derived from a maize not belonging to an available elite maize line or strain of germplasm. In the context of a cross between two maize plants or strains of germplasm, an exotic germplasm is not closely related by descent to the elite germplasm with which it is crossed. Most commonly, the exotic germplasm is not derived from any known elite line of maize, but rather is selected to introduce novel genetic elements (typically novel alleles) into a breeding program.

A "favorable allele" is the allele at a particular locus that confers, or contributes to, an agronomically desirable phenotype, e.g., enhanced resistance to tropical rust, and that allows the identification of plants that agronomically desirable phenotype. A "favorable" allele of a marker is a marker allele that segregates with the favorable phenotype.

"Fragment" is intended to mean a portion of a nucleotide sequence.

Fragments can be used as hybridization probes or PCR primers using methods disclosed herein.

A "genetic map" is a description of genetic linkage relationships among loci on one or more chromosomes (or linkage groups) within a given species, generally depicted in a diagrammatic or tabular form. For each genetic map, distances between loci are measured by how frequently their alleles appear together in a population (i.e. their recombination frequencies). Alleles can be detected using DNA or protein markers, or observable phenotypes. A genetic map is a product of the mapping population, types of markers used, and the polymorphic potential of each marker between different populations. Genetic distances between loci can differ from one genetic map to another. However, information can be correlated from one map to another using common markers. One of ordinary skill in the art can use common marker positions to identify positions of markers and other loci of interest on each individual genetic map. The order of loci should not change between maps, although frequently there are small changes in marker orders due to e.g. markers detecting alternate duplicate loci in different populations, differences in statistical approaches used to order the markers, novel mutation or laboratory error.
A "genetic map location" is a location on a genetic map relative to surrounding genetic markers on the same linkage group where a specified marker can be found within a given species.

"Genetic mapping" is the process of defining the linkage relationships of loci through the use of genetic markers, populations segregating for the markers, and standard genetic principles of recombination frequency.


"Genetic recombination frequency" is the frequency of a crossing over event (recombination) between two genetic loci. Recombination frequency can be observed by following the segregation of markers and/or traits following meiosis.

"Genome" refers to the total DNA, or the entire set of genes, carried by a chromosome or chromosome set.

The term "genotype" is the genetic constitution of an individual (or group of individuals) at one or more genetic loci, as contrasted with the observable trait (the phenotype). Genotype is defined by the allele(s) of one or more known loci that the individual has inherited from its parents. The term genotype can be used to refer to an individual's genetic constitution at a single locus, at multiple loci, or, more generally, the term genotype can be used to refer to an individual's genetic make-up for all the genes in its genome.
"Germplasm" refers to genetic material of or from an individual (e.g., a plant), a group of individuals (e.g., a plant line, variety or family), or a clone derived from a line, variety, species, or culture. The germplasm can be part of an organism or cell, or can be separate from the organism or cell. In general, germplasm provides genetic material with a specific molecular makeup that provides a physical foundation for some or all of the hereditary qualities of an organism or cell culture. As used herein, germplasm includes cells, seed or tissues from which new plants may be grown, or plant parts, such as leaves, stems, pollen, or cells that can be cultured into a whole plant.

A "haplotype" is the genotype of an individual at a plurality of genetic loci, i.e. a combination of alleles. Typically, the genetic loci described by a haplotype are physically and genetically linked, i.e., on the same chromosome segment. The term "haplotype" can refer to a series of polymorphisms with a specific sequence, such as a marker locus, or a series of polymorphisms across multiple sequences, e.g. multiple marker loci.

A "heterotic group" comprises a set of genotypes that perform well when crossed with genotypes from a different heterotic group (Hallauer et al. (1998) Corn breeding, p. 463-564. In G.F. Sprague and J.W. Dudley (ed.) Corn and corn improvement). Inbred lines are classified into heterotic groups, and are further subdivided into families within a heterotic group, based on several criteria such as pedigree, molecular marker-based associations, and performance in hybrid combinations (Smith et al. (1990) Theor. Appl. Gen. 80:833-840). The two most widely used heterotic groups in the United States are referred to as "Iowa Stiff Stalk Synthetic" (BSSS) and "Lancaster" or "Lancaster Sure Crop" (sometimes referred to as NSS, or non-Stiff Stalk).

The term "heterozygous" means a genetic condition wherein different alleles reside at corresponding loci on homologous chromosomes.

The term "homozygous" means a genetic condition wherein identical alleles reside at corresponding loci on homologous chromosomes.

The term "hybrid" refers to the progeny obtained between the crossing of at least two genetically dissimilar parents.

"Hybridization" or "nucleic acid hybridization" refers to the pairing of complementary RNA and DNA strands as well as the pairing of complementary
DNA single strands.

The term "hybridize" means to form base pairs between complementary regions of nucleic acid strands.

An "IBM genetic map" refers to any of following maps: IBM, IBM2, IBM2 neighbors, IBM2 FPC0507, IBM2 2004 neighbors, IBM2 2005 neighbors, or IBM2 2005 neighbors frame. IBM genetic maps are based on a B73 x Mo17 population in which the progeny from the initial cross were random-mated for multiple generations prior to constructing recombinant inbred lines for mapping. Newer versions reflect the addition of genetic and BAC mapped loci as well as enhanced map refinement due to the incorporation of information obtained from other genetic maps.

The term "inbred" refers to a line that has been bred for genetic homogeneity.

The term "indel" refers to an insertion or deletion, wherein one line may be referred to as having an insertion relative to a second line, or the second line may be referred to as having a deletion relative to the first line.

The term "introgression" refers to the transmission of a desired allele of a genetic locus from one genetic background to another. For example, introgression of a desired allele at a specified locus can be transmitted to at least one progeny via a sexual cross between two parents of the same species, where at least one of the parents has the desired allele in its genome. Alternatively, for example, transmission of an allele can occur by recombination between two donor genomes, e.g., in a fused protoplast, where at least one of the donor protoplasts has the desired allele in its genome. The desired allele can be, e.g., a selected allele of a marker, a QTL, a transgene, or the like. In any case, offspring comprising the desired allele can be repeatedly backcrossed to a line having a desired genetic background and selected for the desired allele, to result in the allele becoming fixed in a selected genetic background.

The process of "introgressing" is often referred to as "backcrossing" when the process is repeated two or more times. In introgressing or backcrossing, the "donor" parent refers to the parental plant with the desired gene or locus to be introgressed. The "recipient" parent (used one or more times) or "recurrent" parent (used two or more times) refers to the parental plant into which the gene or locus is being introgressed. For example, see Ragot, M. et al. (1995) Marker-assisted backcrossing: a practical example, inTechniques et Utilisations des Marqueurs
Moleculaires Les Colloques, Vol. 72, pp. 45-56, and Openshaw et al., (1994) Marker-assisted Selection in Backcross Breeding, Analysis of Molecular Marker Data, pp. 41-43. The initial cross gives rise to the F1 generation; the term "BC1" then refers to the second use of the recurrent parent, "BC2" refers to the third use of the recurrent parent, and so on.

As used herein, the term "linkage" is used to describe the degree with which one marker locus is associated with another marker locus or some other locus (for example, a tropical rust locus). The linkage relationship between a molecular marker and a phenotype (for example, enhanced resistance to tropical rust) is given as a "probability" or "adjusted probability". Linkage can be expressed as a desired limit or range. For example, in some embodiments, any marker is linked (genetically and physically) to any other marker when the markers are separated by less than 50, 40, 30, 25, 20, or 15 map units (or cM). In some aspects, it is advantageous to define a bracketed range of linkage, for example, between 10 and 20 cM, between 10 and 30 cM, or between 10 and 40 cM. The more closely a marker is linked to a second locus, the better an indicator for the second locus that marker becomes. Thus, "closely linked loci" such as a marker locus and a second locus display an inter-locus recombination frequency of 10% or less, preferably about 9% or less, still more preferably about 8% or less, yet more preferably about 7% or less, still more preferably about 6% or less, yet more preferably about 5% or less, still more preferably about 4% or less, yet more preferably about 3% or less, and still more preferably about 2% or less. In highly preferred embodiments, the relevant loci display a recombination frequency of about 1% or less, e.g., about 0.75% or less, more preferably about 0.5% or less, or yet more preferably about 0.25% or less.

Two loci that are localized to the same chromosome, and at such a distance that recombination between the two loci occurs at a frequency of less than 10% (e.g., about 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.75%, 0.5%, 0.25%, or less) are also said to be "proximal to" each other. Since one cM is the distance between two markers that show a 1% recombination frequency, any marker is closely linked (genetically and physically) to any other marker that is in close proximity, e.g., at or less than 10 cM distant. Two closely linked markers on the same chromosome can be positioned 9, 8, 7, 6, 5, 4, 3, 2, 1, 0.75, 0.5 or 0.25 cM or less from each other.
The term "linkage disequilibrium" refers to a non-random segregation of genetic loci or traits (or both). In either case, linkage disequilibrium implies that the relevant loci are within sufficient physical proximity along a length of a chromosome so that they segregate together with greater than random (i.e., non-random) frequency (in the case of co-segregating traits, the loci that underlie the traits are in sufficient proximity to each other). Markers that show linkage disequilibrium are considered linked. Linked loci co-segregate more than 50% of the time, e.g., from about 51% to about 100% of the time. In other words, two markers that co-segregate have a recombination frequency of less than 50% (and by definition, are separated by less than 50 cM on the same linkage group.) As used herein, linkage can be between two markers, or alternatively between a marker and a phenotype. A marker locus can be "associated with" (linked to) a trait, e.g., resistance to tropical rust. The degree of linkage of a molecular marker to a phenotypic trait is measured, e.g., as a statistical probability of co-segregation of that molecular marker with the phenotype.

Linkage disequilibrium is most commonly assessed using the measure $r^2$, which is calculated using the formula described by Hill, W.G. and Robertson, A., Theor. Appl. Genet. 38:226-231 (1968). When $r^2 = 1$, complete LD exists between the two marker loci, meaning that the markers have not been separated by recombination and have the same allele frequency. Values for $r^2$ above 1/3 indicate sufficiently strong LD to be useful for mapping (Ardlie et al., Nature Reviews Genetics 3:299-309 (2002)). Hence, alleles are in linkage disequilibrium when $r^2$ values between pairwise marker loci are greater than or equal to 0.33, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or 1.0.

As used herein, "linkage equilibrium" describes a situation where two markers independently segregate, i.e., sort among progeny randomly. Markers that show linkage equilibrium are considered unlinked (whether or not they lie on the same chromosome).

A "locus" is a position on a chromosome where a gene or marker is located.

The "logarithm of odds (LOD) value" or "LOD score" (Risch, Science 255:803-804 (1992)) is used in interval mapping to describe the degree of linkage between two marker loci. A LOD score of three between two markers indicates that linkage is 1000 times more likely than no linkage, while a LOD score of two
indicates that linkage is 100 times more likely than no linkage. LOD scores greater than or equal to two may be used to detect linkage.

"Maize" refers to a plant of the *Zea mays* L. ssp. *mays* and is also known as corn.

The term "maize plant" includes: whole maize plants, maize plant cells, maize plant protoplast, maize plant cell or maize tissue cultures from which maize plants can be regenerated, maize plant callus, and maize plant cells that are intact in maize plants or parts of maize plants, such as maize seeds, maize cobs, maize flowers, maize cotyledons, maize leaves, maize stems, maize buds, maize roots, maize root tips, and the like.

A "marker" is a nucleotide sequence or encoded product thereof (e.g., a protein) used as a point of reference. A marker can be derived from genomic nucleotide sequence or from expressed nucleotide sequences (e.g., from a spliced RNA or a cDNA), or from an encoded polypeptide. The term also refers to nucleic acid sequences complementary to or flanking the marker sequences, such as nucleic acids used as probes or primer pairs capable of amplifying the marker sequence.

Markers corresponding to genetic polymorphisms between members of a population can be detected by methods well-established in the art. These include, e.g., DNA sequencing, PCR-based sequence specific amplification methods, detection of restriction fragment length polymorphisms (RFLP), detection of isozyme markers, detection of polynucleotide polymorphisms by allele specific hybridization (ASH), detection of amplified variable sequences of the plant genome, detection of self-sustained sequence replication, detection of simple sequence repeats (SSRs), detection of single nucleotide polymorphisms (SNPs), or detection of amplified fragment length polymorphisms (AFLPs). Well established methods are also known for the detection of expressed sequence tags (ESTs) and SSR markers derived from EST sequences and randomly amplified polymorphic DNA (RAPD).

A "marker allele", alternatively an "allele of a marker locus", can refer to one of a plurality of polymorphic nucleotide sequences found at a marker locus in a population that is polymorphic for the marker locus.

"Marker assisted selection" (of MAS) is a process by which individual plants are selected based on marker genotypes.
"Marker assisted counter-selection" is a process by which marker genotypes are used to identify plants that will not be selected, allowing them to be removed from a breeding program or planting.

A "marker locus" is a specific chromosome location in the genome of a species where a specific marker can be found. A marker locus can be used to track the presence of a second linked locus, e.g., a linked locus that encodes or contributes to expression of a phenotypic trait. For example, a marker locus can be used to monitor segregation of alleles at a locus, such as a gene or QTL, that are genetically or physically linked to the marker locus.

A "marker probe" is a nucleic acid sequence or molecule that can be used to identify the presence of a marker locus, e.g., a nucleic acid probe that is complementary to a marker locus sequence, through nucleic acid hybridization. Marker probes comprising 30 or more contiguous nucleotides of the marker locus ("all or a portion" of the marker locus sequence) may be used for nucleic acid hybridization. Alternatively, in some aspects, a marker probe refers to a probe of any type that is able to distinguish (i.e., genotype) the particular allele that is present at a marker locus. Nucleic acids are "complementary" when they specifically "hybridize", or pair, in solution, e.g., according to Watson-Crick base pairing rules.

The term "molecular marker" may be used to refer to a genetic marker, as defined above, or an encoded product thereof (e.g., a protein) used as a point of reference when identifying a linked locus. A marker can be derived from genomic nucleotide sequences or from expressed nucleotide sequences (e.g., from a spliced RNA, a cDNA, etc.), or from an encoded polypeptide. The term also refers to nucleic acid sequences complementary to or flanking the marker sequences, such as nucleic acids used as probes or primer pairs capable of amplifying the marker sequence. A "molecular marker probe" is a nucleic acid sequence or molecule that can be used to identify the presence of a marker locus, e.g., a nucleic acid probe that is complementary to a marker locus sequence. Alternatively, in some aspects, a marker probe refers to a probe of any type that is able to distinguish (i.e., genotype) the particular allele that is present at a marker locus. Nucleic acids are "complementary" when they specifically hybridize in solution, e.g., according to Watson-Crick base pairing rules. Some of the markers described herein are also referred to as hybridization markers when located on an indel region, such as the
non-collinear region described herein. This is because the insertion region is, by
definition, a polymorphism vis a vis a plant without the insertion. Thus, the marker
need only indicate whether the indel region is present or absent. Any suitable
marker detection technology may be used to identify such a hybridization marker,
e.g. SNP technology is used in the examples provided herein.

"Tropical rust" is the disease caused by the pathogen Physopella zeae
(Mains) Cummins & Ramachar (syn. Angiopsora zeae Mains). The disease is
characterized by the formation of small round yellow pustules on the upper surface
of the corn leaf. These uredial pustules are often found in small groups and the leaf
epidermal layer covers the developing urediniospores. The obovoid to ellipsoid
shaped urediniospores are released through a small slit or pore that forms in the
epidermal layer. While the urediniospores are nearly colorless their released
urediniospores give the pustules a white or creamy appearance. Some maize
genotypes display pustules with a darker coloration (reddish to purplish) which
accentuates the white/creamy urediniospores vs. a more traditional. A telial stage,
with blister like appearance can also develop following uredial stage formation. The
teliospores (brown to black in color) can develop within the telia which forms around
the existing uredial pustules. (Donald G. White, ed. 1999. Compendium of corn

"Southern rust" is the disease caused by the pathogen Puccinia polysora
Underw.. The disease is characterized by small round yellow pustules that form
primarily on the upper surface of the leaf, but occasionally break through to the
lower leaf surface with uredial sporulation most often found adjacent to the leaf
midrib. These uredial pustules contain the obovoid to ellipsoid shaped
urediniospores, which typically are orange to reddish orange in coloration. The
pustules often are round to oval in shape and become very numerous on the leaf.
This pathogen can also form uredial pustules on the ear husk, ear shank and leaf
sheaths. A telial stage is known to exist, with dark brown to black teliospores
forming in telial which found in a semi-circle to circle around existing uredia.

"Nucleotide sequence", "polynucleotide", "nucleic acid sequence", and
"nucleic acid fragment" are used interchangeably and refer to a polymer of RNA or
DNA that is single- or double-stranded, optionally containing synthetic, non-natural
or altered nucleotide bases. A "nucleotide" is a monomeric unit from which DNA or
RNA polymers are constructed, and consists of a purine or pyrimidine base, a pentose, and a phosphoric acid group. Nucleotides (usually found in their 5\'-monophosphate form) are referred to by their single letter designation as follows: "A" for adenylate or deoxyadenylate (for RNA or DNA, respectively), "C" for cytidylate or deoxycytidylate, "G" for guanylate or deoxyguanylate, "U" for uridylate, "T" for deoxthymidylate, "R" for purines (A or G), "Y" for pyrimidines (C or T), "K" for G or T, "H" for A or C or T, "I" for inosine, and "N" for any nucleotide.

A phenogram is a diagram depicting taxonomic relationships among organisms based on overall similarity of many characteristics without regard to evolutionary history or assumed significance of specific characters, usually generated by a computer.

The terms "phenotype", or "phenotypic trait" or "trait" refers to one or more trait of an organism. The phenotype can be observable to the naked eye, or by any other means of evaluation known in the art, e.g., microscopy, biochemical analysis, or an electromechanical assay. In some cases, a phenotype is directly controlled by a single gene or genetic locus, i.e., a "single gene trait". In other cases, a phenotype is the result of several genes.

"Phylogenetic trees" are diagrams showing the inferred evolutionary relationships among various biological species or other entities based upon similarities and differences in their physical and/or genetic characteristics. They can be constructed using a variety of methods including but not limited to the distance-matrix methods such as neighbor-joining or UPGMA, which calculate genetic distance from multiple sequence alignments.

A "physical map" of the genome is a map showing the linear order of identifiable landmarks (including genes, markers, etc.) on chromosome DNA. However, in contrast to genetic maps, the distances between landmarks are absolute (for example, measured in base pairs or isolated and overlapping contiguous genetic fragments) and not based on genetic recombination.

A "plant" can be a whole plant, any part thereof, or a cell or tissue culture derived from a plant. Thus, the term "plant" can refer to any of: whole plants, plant components or organs (e.g., leaves, stems, roots, etc.), plant tissues, seeds, plant cells, and/or progeny of the same. A plant cell is a cell of a plant, taken from a plant, or derived through culture from a cell taken from a plant.
A "polymorphism" is a variation in the DNA that is too common to be due merely to new mutation. A polymorphism must have a frequency of at least 1% in a population. A polymorphism can be a single nucleotide polymorphism, or SNP, or an insertion/deletion polymorphism, also referred to herein as an "indel". The "probability value" or "p-value" is the statistical likelihood that the particular combination of a phenotype and the presence or absence of a particular marker allele is random. Thus, the lower the probability score, the greater the likelihood that a phenotype and a particular marker will co-segregate. In some aspects, the probability score is considered "significant" or "nonsignificant". In some embodiments, a probability score of 0.05 (p=0.05, or a 5% probability) of random assortment is considered a significant indication of co-segregation. However, an acceptable probability can be any probability of less than 50% (p=0.5). For example, a significant probability can be less than 0.25, less than 0.20, less than 0.15, less than 0.1, less than 0.05, less than 0.01, or less than 0.001.

Each "PHM" marker represents two sets of primers (external and internal) that when used in a nested PCR, amplify a specific piece of DNA. The external set is used in the first round of PCR, after which the internal sequences are used for a second round of PCR on the products of the first round. This increases the specificity of the reaction. All of the PHM markers described herein are listed in Table 2, and the annealing temperature for these primers is 55°C.

A "production marker" or "production SNP marker" is a marker that has been developed for high-throughput purposes. Production SNP markers were developed for specific polymorphisms identified using PHM markers and the nested PCR analysis (see, for example, PHM1 192-26-U in Table 1). The production SNP markers were designed for use with the Invader Plus® (Third Wave Technologies) platform.

A "reference sequence" is a defined sequence used as a basis for sequence comparison. The reference sequence is obtained by genotyping a number of lines at the locus, aligning the nucleotide sequences in a sequence alignment program (e.g. Sequencher), and then obtaining the consensus sequence of the alignment. Hence, a reference sequence identifies the polymorphisms in alleles at a locus. A reference sequence may not be a copy of an actual DNA sequence; however, it is useful for designing primers and probes for actual polymorphisms in the locus.
The term "progeny" refers to the offspring generated from a cross. A "progeny plant" is generated from a cross between two plants.

The term "quantitative trait locus" or "QTL" refers to a region of DNA that is associated with the differential expression of a phenotypic trait in at least one genetic background, e.g., in at least one breeding population. QTLs are closely linked to the gene or genes that underlie the trait in question.

A "topcross test" is a progeny test derived by crossing each parent with the same tester, usually a homozygous line. The parent being tested can be an open-pollinated variety, a cross, or an inbred line.

The phrase "under stringent conditions" refers to conditions under which a probe or polynucleotide will hybridize to a specific nucleic acid sequence, typically in a complex mixture of nucleic acids, but to essentially no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. An "unfavorable allele" of a marker is a marker allele that segregates with the unfavorable plant phenotype, therefore providing the benefit of identifying plants that can be removed from a breeding program or planting.

Longer sequences hybridize specifically at higher temperatures. Generally, stringent conditions are selected to be about 3-5°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at Tm, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3, and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, preferably 10 times background hybridization. Exemplary stringent hybridization conditions are often: 50% formamide, 5x SSC, and 1% SDS, incubating at 42°C, or, 5x SSC, 1% SDS, incubating at 65°C, with wash in 0.2x SSC, and 0.1% SDS at 65°C. For PCR, a temperature of about 36°C is typical for
low stringency amplification, although annealing temperatures may vary between about 50°C and 65°C, depending on primer length. Additional guidelines for determining hybridization parameters are provided in numerous references. Sequence alignments and percent identity calculations may be determined using a variety of comparison methods designed to detect homologous sequences including, but not limited to, the MEGALIGN® program of the LASERGENE® bioinformatics computing suite (DNASTAR® Inc., Madison, WI). Unless stated otherwise, multiple alignment of the sequences provided herein were performed using the Clustal V method of alignment (Higgins and Sharp, CABIOS. 5:1 51-153 (1989)) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments and calculation of percent identity of protein sequences using the Clustal V method are KTUPLE=1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. For nucleic acids these parameters are KTUPLE=2, GAP PENALTY=5, WINDOW=4 and DIAGONALS SAVED=4. After alignment of the sequences, using the Clustal V program, it is possible to obtain "percent identity" and "divergence" values by viewing the "sequence distances" table on the same program; unless stated otherwise, percent identities and divergences provided and claimed herein were calculated in this manner.

The "Clustal V method of alignment" corresponds to the alignment method labeled Clustal V (described by Higgins and Sharp, CABIOS. 5:151-153 (1989); Higgins, D.G. et al. (1992) Comput. Appl. Biosci. 8:189-191 ) and found in the MegAlign™ program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). For multiple alignments, the default values correspond to GAP PENALTY=10 and GAP LENGTH PENALTY=10. Default parameters for pairwise alignments and calculation of percent identity of protein sequences using the Clustal method are KTUPLE=1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. For nucleic acids these parameters are KTUPLE=2, GAP PENALTY=5, WINDOW=4 and DIAGONALS SAVED=4. After alignment of the sequences using the Clustal V program, it is possible to obtain a "percent identity" by viewing the "sequence distances" table in the same program.

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook, J., Fritsch, E.F.

Before describing the present invention in detail, it should be understood that this invention is not limited to particular embodiments. It also should be understood that the terminology used herein is for the purpose of describing particular embodiments, and is not intended to be limiting. As used herein and in the appended claims, terms in the singular and the singular forms "a", "an" and "the", for example, include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "plant", "the plant" or "a plant" also includes a plurality of plants. Depending on the context, use of the term "plant" can also include genetically similar or identical progeny of that plant. The use of the term "a nucleic acid" optionally includes many copies of that nucleic acid molecule.

**Tropical rust resistance**

Tropical rust resistance is a fungal disease of maize caused by the pathogen *Physopella zeae.* The identification of molecular markers and alleles associated with tropical rust resistance allows selection for resistance based solely on the genetic composition of the progeny. Methods for identifying and selecting maize plants with enhanced resistance to tropical rust through the evaluation of genetic composition (as assessed using molecular markers and their alleles) are presented herein.

**Genetic mapping**

It has been recognized for quite some time that specific genetic loci correlating with particular phenotypes, such as resistance to tropical rust, can be mapped in an organism’s genome. The plant breeder can advantageously use molecular markers to identify desired individuals by identifying marker alleles that show a statistically significant probability of co-segregation with a desired phenotype, manifested as linkage disequilibrium. By identifying a molecular marker or clusters of molecular markers that co-segregate with a trait of interest, the breeder is able to rapidly select a desired phenotype by selecting for the proper molecular marker allele (a process called marker-assisted selection, or MAS). Such markers could also be used by breeders to design genotypes in silico and to practice whole genome selection.

A variety of methods well known in the art are available for detecting
molecular markers or clusters of molecular markers that co-segregate with a quantitative trait such as resistance to tropical rust. The basic idea underlying these methods is the detection of markers, for which alternative genotypes (or alleles) have significantly different average phenotypes. Thus, one makes a comparison among marker loci of the magnitude of difference among alternative genotypes (or alleles) or the level of significance of that difference. Trait genes are inferred to be located nearest the marker(s) that have the greatest associated genotypic difference.

Two such methods that can be used to detect loci of interest are: 1) Population-based association analysis and 2) Pedigree-based association analysis (or traditional linkage mapping). In a population-based association analysis, lines are obtained from pre-existing populations with multiple founders, e.g. elite breeding lines. Population-based association analyses rely on the decay of linkage disequilibrium (LD) and the idea that in an unstructured population, only correlations between genes controlling a trait of interest and markers closely linked to the those genes will remain after so many generations of random mating. In reality, most pre-existing populations have population substructure. Thus, the use of a structured association approach helps to control population structure by allocating individuals to populations using data obtained from markers randomly distributed across the genome, thereby minimizing disequilibrium due to population structure within the individual populations (also called subpopulations). The phenotypic values are compared to the genotypes (alleles) at each marker locus for each line in the subpopulation. A significant marker-trait association indicates the close proximity between the marker locus and one or more genetic loci that are involved in the expression of that trait.

The same principles underlie the pedigree-based association analyses (also referred to as traditional linkage analysis); however, LD is generated by creating a population from a small number of founders. The founders are selected to maximize the level of polymorphism within the constructed population, and polymorphic sites are assessed for their level of cosegregation with a given phenotype. A number of statistical methods have been used to identify significant marker-trait associations. One such method is an interval mapping approach (Lander and Botstein, *Genetics* 121:185-199 (1989), in which each of many
positions along a genetic map (say at 1 cM intervals) is tested for the likelihood that a gene controlling a trait of interest is located at that position. The genotype/phenotype data are used to calculate for each test position a LOD score (log of likelihood ratio). When the LOD score exceeds a threshold value, there is significant evidence for the location of a gene controlling the trait of interest at that position on the genetic map (which will fall between two particular marker loci).

The present invention provides molecular marker loci that demonstrate cosegregation with resistance to tropical rust as determined by traditional linkage analysis (FIG. 3). Detection of these marker loci or additional linked marker loci can be used in marker assisted maize breeding programs to produce plants with enhanced resistance to tropical rust or to eliminate plants with an unfavorable tropical rust phenotype from breeding programs or planting.

Markers associated with resistance to tropical rust

Markers associated with resistance to tropical rust are identified herein, as are marker alleles associated with either increased (enhanced) or decreased resistance to tropical rust. The methods involve detecting the presence of one or more marker alleles associated with the enhanced resistance in a maize plant or germplasm. The maize plant can be a hybrid or an inbred.

The marker locus can be selected from any of the marker loci provided herein, including but not limited to the SNP production markers PHM1 192-26-U, PHM1 192-4-U, C00435-802-U, C00436-801-U, PHM187-7-U, C00423-801-U, PHM5028-24-U, PHM13818-15-U, PHM15721-39-U, PHM15721-180-U, C00441-801-U, C00441-802-U, PHM4370-19-U, PHM731-107-U, C00071-01-U, PHM8249-21-U, C00428-801-U, PHM1 8427-1 3-U, PHM9535-1 0-U, PHM9535-6-U, PHM9535-7-U, and PHM4003-13-U; the PHM markers PHM15590, PHM13818, PHM1 192, PHM187, PHM5028, PHM4370, PHM731, and PHM15721; Sub2e, Sub9d, Sub19c, Sub23m, C06621-1-K2, and C06621-1-K4, as well as any other marker linked to these markers.

Physical map location of the interval comprising the tropical rust resistance gene

The genetic elements or genes located on a contiguous linear span of genomic DNA on a single chromosome are physically linked.

The present invention provides molecular marker loci on an area of chromosome 10 defined by and including PHM15590 and PHM1 5721, thereby
delineating a region comprising a gene that confers resistance to tropical rust. PHM15590 is located on BAC C0497L12, and PHM15721 is located on b0191 E02. Any polynucleotide that can hybridize or assemble to the contiguous DNA between and including SEQ ID NO:89 (the reference sequence for PHM15590), or a nucleotide sequence that is 95% identical to SEQ ID NO:89 based on the Clustal V method of alignment, and SEQ ID NO:96 (the reference sequence for PHM15721 or a nucleotide sequence that is 95% identical to SEQ ID NO:96 based on the Clustal V method of alignment, and that is associated with tropical rust resistance can be used as a marker for tropical rust. This physical region encompasses marker loci that are shown herein to be associated with the tropical rust resistance trait.

FIG.1 shows the physical map arrangement of the sequenced B73 BACs that make up the contiguous stretch of DNA between and including BAC c0497L12 and BAC C0352E09. The gaps (represented by open spaces) are not gaps in the contiguous stretch of DNA per se but are areas where genome sequencing information is incomplete.

Linkage relationships

A common measure of linkage is the frequency with which traits cosegregate. This can be expressed as a percentage of cosegregation (recombination frequency) or in centiMorgans (cM). The cM is a unit of measure of genetic recombination frequency. One cM is equal to a 1% chance that a trait at one genetic locus will be separated from a trait at another locus due to crossing over in a single generation (meaning the traits segregate together 99% of the time). Because chromosomal distance is approximately proportional to the frequency of crossing over events between traits, there is an approximate physical distance that correlates with recombination frequency.

Marker loci are themselves traits and can be assessed according to standard linkage analysis by tracking the marker loci during segregation. Thus, one cM is equal to a 1% chance that a marker locus will be separated from another locus, due to crossing over in a single generation.

The closer a marker is to gene controlling a trait of interest, the more effective and advantageous that marker is as an indicator for the desired trait. Closely linked loci display an inter-locus cross-over frequency of about 10% or less, preferably about 9% or less, still more preferably about 8% or less, yet more preferably about
7% or less, still more preferably about 6% or less, yet more preferably about 5% or
less, still more preferably about 4% or less, yet more preferably about 3% or less,
and still more preferably about 2% or less. In highly preferred embodiments, the
relevant loci (e.g., a marker locus and a target locus) display a recombination
frequency of about 1% or less, e.g., about 0.75% or less, more preferably about
0.5% or less, or yet more preferably about 0.25% or less. Thus, the loci are about
10 cM, 9 cM, 8 cM, 7 cM, 6 cM, 5 cM, 4 cM, 3 cM, 2 cM, 1 cM, 0.75 cM, 0.5 cM or
0.25 cM or less apart. Put another way, two loci that are localized to the same
chromosome, and at such a distance that recombination between the two loci
occurs at a frequency of less than 10% (e.g., about 9%, 8%, 7%, 6%, 5%, 4%, 3%,
2%, 1%, 0.75%, 0.5%, 0.25%, or less) are said to be "proximal to" each other.

Although particular marker alleles can show co-segregation with the tropical
rust resistance phenotype, it is important to note that the marker locus is not
necessarily part of a gene or QTL locus responsible for the expression of the
tropical rust resistance phenotype. For example, it is not a requirement that the
marker polynucleotide sequence be part of a gene that imparts tropical rust
resistance (for example, be part of the gene open reading frame). The association
between a specific marker allele with either a favorable or unfavorable tropical rust
resistance phenotype is due to the original "coupling" linkage phase between the
marker allele and the founder allele in the ancestral maize line. Eventually, with
repeated recombination, crossing over events between the marker and the genetic
locus can change this orientation. For this reason, the favorable marker allele may
change depending on the linkage phase that exists within the resistant parent used
to create segregating populations. This does not change the fact that the marker
can be used to monitor segregation of the phenotype. It only changes which marker
allele is considered favorable in a given segregating population.

Markers provided herein can be used to predict the state of the tropical rust
resistance trait in a maize plant. This includes any marker within 20, 10, 9, 8, 7, 6,
5, 4, 3, 2, 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, or 0.1 cM of any of the SNP
production markers PHM1 192-26-U, PHM1 192-4-U, C00435-802-U, C00436-801-U,
PHM15721-180-U, C00441-801-U, C00441-802-U, PHM4370-1 9-U, PHM731-107-
U, C00071-01-U, PHM8249-21-U, C00428-801-U, PHM1 8427-1 3-U, PHM9535-1 0-
U, PHM9535-6-U, PHM9535-7-U, and PHM4003-13-U; the PHM markers
PHM15590, PHM13818, PHM1 192, PHM1 87, PHM5028, PHM4370, PHM731, and
PHM15721; and the other markers identified herein, Sub2e, Sub9d, Sub19c,
Sub23m, C06621-1-K2, and C06621-1-K4.

5 Chromosomal intervals

A variety of methods well known in the art are available for identifying
chromosomal intervals. The boundaries of such chromosomal intervals are drawn
to encompass markers that will be linked to the gene controlling the trait of interest.
In other words, the chromosomal interval is drawn such that any marker that lies
within that interval (including the terminal markers that define the boundaries of the
interval) can be used as markers for tropical rust resistance.

Chromosomal intervals encompassing markers that co-segregate with
tropical rust resistance are provided. These intervals are located on chromosome
10 and may be defined by and include:

(i) PHM15590 and PHM9535;
(ii) PHM15590 and PHM1 5721;
(iii) C00441 and c00428;
(iv) PHM731 and PHM15721; or
(v) C00071 and PHM731.

Any marker located within any of these intervals can find use as a marker for
tropical rust resistance.

Chromosomal intervals can also be defined by markers that are linked to
(show linkage disequilibrium with) a QTL marker, and $r^2$ is a common measure of
linkage disequilibrium (LD) in the context of association studies. If the $r^2$ value of LD
between a chromosome 10 marker locus lying within the interval of PHM15590 and
PHM9535, for example, and another chromosome 10 marker locus in close
proximity is greater than 1/3 (Ardlie et al., Nature Reviews Genetics 3:299-309
(2002)), the loci are in linkage disequilibrium with one another.

Marker alleles and haplotypic combinations

A marker of the invention can also be a combination of alleles at one or more
marker loci (i.e. a haplotype). The alleles described below could be used alone or in
combination to identify and select maize plants with enhanced tropical rust
resistance.
Favorable alleles associated with enhanced tropical rust resistance have been identified herein. One such allele is a "T" deletion at position 16 of PHMTR (SEQ ID NO:155). FIG. 7 shows a part of the reference sequence (top) obtained by the genotyping of maize lines resistant and susceptible to tropical rust using PCR primers (SEQ ID NO: 133 and 134) designed for clone ID Ct9050c064G1 1c (Table 9). SEQ ID NOs: 137-142 represent amplicons obtained from resistant lines, and SEQ ID NOs: 143-154 represent amplicons obtained from susceptible lines. The area highlighted in grey represents a 21 bp-region of the reference sequence (referred to as PHMTR; SEQ ID NO:155). Maize lines having a T-deletion at bp 16 of PHMTR (indicated by the arrow) all showed enhanced resistance to tropical rust. Maize lines having an intact PHMTR region all showed sensitivity to tropical rust.

Tables 7 and 8 also show chromosome 10 markers that have been successfully used in combination to convert susceptible inbreds into resistant inbreds using PHS6Y as the source. The alleles possessed by PHS6Y at each of the markers can be used in combination (as a haplotype) to identify and select plants with enhanced resistance to tropical rust.

While a haplotype associated with enhanced resistant to tropical rust may comprise any of the favorable alleles described herein (including the "T" deletion at position 16 of PHMTR and any of the marker alleles possessed by the resistant line PHS6Y in Tables 7 and 8), the "GAG" haplotype at positions 337-339 of reference sequence SEQ ID NO:167 was shown to be associated with enhanced resistance to tropical rust and can be used in a marker assisted selection program to select for maize plants with enhanced resistance to tropical rust.

The skilled artisan would expect that there might be additional polymorphic sites at marker loci in and around the chromosome 10 markers identified herein, wherein one or more polymorphic sites is in linkage disequilibrium (LD) with an allele at one or more of the polymorphic sites in the haplotype. Two particular alleles at different polymorphic sites are said to be in LD if the presence of the allele at one of the sites tends to predict the presence of the allele at the other site on the same chromosome (Stevens, Mol. Diag. 4:309-17 (1999)).

The skilled artisan would understand that allelic frequency (and hence, haplotype frequency) can differ from one germplasm pool to another. Germplasm pools vary due to maturity differences, heterotic groupings, geographical
distribution, etc. As a result, SNPs and other polymorphisms may not be informative in some germplasm pools.

**Marker assisted selection**

Molecular markers can be used in a variety of plant breeding applications (e.g. see Staub et al. (1996) *Hortscience* 31: 729-741; Tanksley (1983) *Plant Molecular Biology Reporter*. 1: 3-8). One of the main areas of interest is to increase the efficiency of backcrossing and introgressing genes using marker-assisted selection (MAS). A molecular marker that demonstrates linkage with a locus affecting a desired phenotypic trait provides a useful tool for the selection of the trait in a plant population. This is particularly true where the phenotype is hard to assay, e.g. many disease resistance traits, or, occurs at a late stage in plant development, e.g. kernel characteristics. Since DNA marker assays are less laborious and take up less physical space than field phenotyping, much larger populations can be assayed, increasing the chances of finding a recombinant with the target segment from the donor line moved to the recipient line. The closer the linkage, the more useful the marker, as recombination is less likely to occur between the marker and the gene causing the trait, which can result in false positives. Having flanking markers decreases the chances that false positive selection will occur as a double recombination event would be needed. The ideal situation is to have a marker in the gene itself, so that recombination cannot occur between the marker and the gene. Such a marker is called a "perfect marker".

When a gene is introgressed by MAS, it is not only the gene that is introduced but also the flanking regions (Gepts. (2002). *Crop Sci*; 42: 1780-1790). This is referred to as "linkage drag." In the case where the donor plant is highly unrelated to the recipient plant, these flanking regions carry additional genes that may code for agronomically undesirable traits. This "linkage drag" may also result in reduced yield or other negative agronomic characteristics even after multiple cycles of backcrossing into the elite maize line. This is also sometimes referred to as "yield drag." The size of the flanking region can be decreased by additional backcrossing, although this is not always successful, as breeders do not have control over the size of the region or the recombination breakpoints (Young et al. (1998) *Genetics* 120:579-585). In classical breeding it is usually only by chance that recombinations are selected that contribute to a reduction in the size of the donor segment.
(Tanksley et al. (1989). *Biotechnology* 7: 257-264). Even after 20 backcrosses in backcrosses of this type, one may expect to find a sizeable piece of the donor chromosome still linked to the gene being selected. With markers however, it is possible to select those rare individuals that have experienced recombination near the gene of interest. In 150 backcross plants, there is a 95% chance that at least one plant will have experienced a crossover within 1 cM of the gene, based on a single meiosis map distance. Markers will allow unequivocal identification of those individuals. With one additional backcross of 300 plants, there would be a 95% chance of a crossover within 1 cM single meiosis map distance of the other side of the gene, generating a segment around the target gene of less than 2 cM based on a single meiosis map distance. This can be accomplished in two generations with markers, while it would have required on average 100 generations without markers (See Tanksley et al., supra). When the exact location of a gene is known, a series of flanking markers surrounding the gene can be utilized to select for recombinations in different population sizes. For example, in smaller population sizes, recombinations may be expected further away from the gene, so more distal flanking markers would be required to detect the recombination.

The availability of integrated linkage maps of the maize genome containing increasing densities of public maize markers has facilitated maize genetic mapping and MAS. See, *e.g.* the IBM2 Neighbors maps, which are available online on the MaizeGDB website.

The key components to the implementation of MAS are: (i) Defining the population within which the marker-trait association will be determined, which can be a segregating population, or a random or structured population; (ii) monitoring the segregation or association of polymorphic markers relative to the trait, and determining linkage or association using statistical methods; (iii) defining a set of desirable markers based on the results of the statistical analysis, and (iv) the use and/or extrapolation of this information to the current set of breeding germplasm to enable marker-based selection decisions to be made. The markers described in this disclosure, as well as other marker types such as SSRs and FLPs, can be used in marker assisted selection protocols.

SSRs can be defined as relatively short runs of tandemly repeated DNA with lengths of 6 bp or less (Tautz (1989) *Nucleic Acid Research* 17: 6463-6471; Wang et
al. (1994) *Theoretical and Applied Genetics*, 88:1-6) Polymorphisms arise due to
variation in the number of repeat units, probably caused by slippage during DNA
repeat length may be detected by designing PCR primers to the conserved non-
SSRs are highly suited to mapping and MAS as they are multi-allelic, codominant,
reproducible and amenable to high throughput automation (Rafalski et al. (1996)

Various types of SSR markers can be generated, and SSR profiles from
resistant lines can be obtained by gel electrophoresis of the amplification products.
Scoring of marker genotype is based on the size of the amplified fragment. An SSR
service for maize is available to the public on a contractual basis by DNA
Landmarks in Saint-Jean-sur-Richelieu, Quebec, Canada.

Various types of FLP markers can also be generated. Most commonly,
amplification primers are used to generate fragment length polymorphisms. Such
FLP markers are in many ways similar to SSR markers, except that the region
amplified by the primers is not typically a highly repetitive region. Still, the amplified
region, or amplicon, will have sufficient variability among germplasm, often due to
insertions or deletions, such that the fragments generated by the amplification
primers can be distinguished among polymorphic individuals, and such indels are
known to occur frequently in maize (Bhattaramakki et al. (2002). *Plant Mol Biol* 48,
539-547; Rafalski (2002b), supra).

SNP markers detect single base pair nucleotide substitutions. Of all the
molecular marker types, SNPs are the most abundant, thus having the potential to
provide the highest genetic map resolution (Bhattaramakki et al. 2002 *Plant Molecular
Biology* 48:539-547). SNPs can be assayed at an even higher level of throughput
than SSRs, in a so-called 'ultra-high-throughput' fashion, as they do not require
large amounts of DNA and automation of the assay may be straight-forward. SNPs
also have the promise of being relatively low-cost systems. These three factors
together make SNPs highly attractive for use in MAS. Several methods are
available for SNP genotyping, including but not limited to, hybridization, primer
extension, oligonucleotide ligation, nuclease cleavage, minisequencing and coded

A number of SNPs together within a sequence, or across linked sequences, can be used to describe a haplotype for any particular genotype (Ching et al. (2002), *BMC Genet.* 3:19 pp Gupta et al. 2001 , Rafalski (2002b), *Plant Science* 162:329-333). Haplotypes can be more informative than single SNPs and can be more descriptive of any particular genotype. For example, a single SNP may be allele `T` for a specific line or variety with enhanced resistance to tropical rust, but the allele `T` might also occur in the maize breeding population being utilized for recurrent parents. In this case, a haplotype, e.g. a series of alleles at linked SNP markers, may be more informative. Once a unique haplotype has been assigned to a donor chromosomal region, that haplotype can be used in that population or any subset thereof to determine whether an individual has a particular gene. See, for example, WO2003054229. Using automated high throughput marker detection platforms known to those of ordinary skill in the art makes this process highly efficient and effective.

Many of the primers listed in Table 2 can readily be used as FLP markers to select for the gene locus or QTL on chromosome 10 controlling resistance to tropical rust, owing to the presence of insertions/deletion polymorphisms. These primers can also be used to convert these markers to SNP or other structurally similar or functionally equivalent markers (SSRs, CAPs, indels, etc), in the same regions. One very productive approach for SNP conversion is described by Rafalski (2002a) *Current opinion in plant biology* 5 (2): 94-100 and also Rafalski (2002b) *Plant Science* 162: 329-333. Using PCR, the primers are used to amplify DNA segments from individuals (preferably inbred) that represent the diversity in the population of interest. The PCR products are sequenced directly in one or both
directions. The resulting sequences are aligned and polymorphisms are identified. The polymorphisms are not limited to single nucleotide polymorphisms (SNPs), but also include indels, CAPS, SSRs, and VNTRs (variable number of tandem repeats). Specifically with respect to the fine map information described herein, one can readily use the information provided herein to obtain additional polymorphic SNPs (and other markers) within the region amplified by the primers listed in this disclosure. Markers within the described map region can be hybridized to BACs or other genomic libraries, or electronically aligned with genome sequences, to find new sequences in the same approximate location as the described markers.

In addition to SSR's, FLPs and SNPs, as described above, other types of molecular markers are also widely used, including but not limited to expressed sequence tags (ESTs), SSR markers derived from EST sequences, randomly amplified polymorphic DNA (RAPD), and other nucleic acid based markers. Isozyme profiles and linked morphological characteristics can, in some cases, also be indirectly used as markers. Even though they do not directly detect DNA differences, they are often influenced by specific genetic differences. However, markers that detect DNA variation are far more numerous and polymorphic than isozyme or morphological markers (Tanksley (1983) Plant Molecular Biology Reporter 1:3-8).

Sequence alignments or contigs may also be used to find sequences upstream or downstream of the specific markers listed herein. These new sequences, close to the markers described herein, are then used to discover and develop functionally equivalent markers. For example, different physical and/or genetic maps are aligned to locate equivalent markers not described within this disclosure but that are within similar regions. These maps may be within the maize species, or even across other species that have been genetically or physically aligned with maize, such as rice, wheat, barley or sorghum.

In general, MAS uses polymorphic markers that have been identified as having a significant likelihood of co-segregation with tropical rust resistance. Such markers are presumed to map near a gene or genes that give the plant its tropical rust resistance phenotype, and are considered indicators for the desired trait, or markers. Plants are tested for the presence of a desired allele in the marker, and plants containing a desired genotype at one or more loci are expected to transfer
the desired genotype, along with a desired phenotype, to their progeny.

The markers and intervals presented herein find use in MAS to select plants that demonstrate enhanced resistance to tropical rust.

Methods for selection can involve detecting the presence (or absence) of either an identified marker allele or an unknown marker allele that is linked to and associated with an identified marker allele in a maize plant or germplasm and then selecting the maize plant or germplasm based on the allele detected. Favorable alleles identified herein that could be detected in MAS include: the "T" deletion at position 16 of PHMTR and any of the marker alleles possessed by PHS6Y in Tables 7 and 8. In addition, favorable haplotypes, such as the "GAG" haplotype at positions 337-339 of reference sequence SEQ ID NO:167, can also be used in MAS to introduce enhanced resistance to tropical rust into susceptible maize lines or varieties.

Usefulness of MAS for Enhancing Resistance to Tropical Rust in Maize

Maize plant breeders desire combinations of desired genetic loci, such as those marker alleles associated with enhanced resistance to tropical rust, with genes for high yield and other desirable traits to develop improved maize varieties. Screening large numbers of samples by non-molecular methods (e.g., trait evaluation in maize plants) can be expensive, time consuming, and unreliable. Use of the polymorphic markers described herein, when genetically-linked to resistance to tropical rust loci, provide an effective method for selecting varieties with enhanced resistance to tropical rust in breeding programs. For example, one advantage of marker-assisted selection over field evaluations for the selection of plants that have enhanced resistance to tropical rust is that MAS can be done at any time of year, regardless of the growing season. Moreover, environmental effects are largely irrelevant to marker-assisted selection.

Another use of MAS in plant breeding is to assist the recovery of the recurrent parent genotype by backcross breeding. Backcross breeding is the process of crossing a progeny back to one of its parents or parent lines. Backcrossing is usually done for the purpose of introgressing one or a few loci from a donor parent (e.g., a parent comprising enhanced resistance to tropical rust marker loci) into an otherwise desirable genetic background from the recurrent parent (e.g., an otherwise high yielding maize line). The more cycles of
backcrossing that are done, the greater the genetic contribution of the recurrent
parent to the resulting introgressed variety. This is often necessary, because plants
may be otherwise undesirable, e.g., due to low yield, low fecundity, or the like. In
contrast, strains which are the result of intensive breeding programs may have
excellent yield, fecundity or the like, merely being deficient in one desired trait such
as resistance to tropical rust.

MAS can increase the efficiency of an introgression or backcrossing effort
aimed at introducing enhanced resistance to tropical rust into a desired (typically
high yielding) background. In marker assisted backcrossing of specific markers
from a donor source, e.g., to an elite or exotic genetic background, one selects
among backcross progeny for the donor trait and then uses repeated backcrossing
to the elite or exotic line to reconstitute as much of the elite/exotic background's
genome as possible.

Multiple stages cluster methodology

A multiple stages cluster methodology can be used to direct primer design
into non-random variation. This method uses phylogenetic trees and a sequential
alignment process to identify unique regions containing allelic variations exclusive to
lines with a desired trait. If any given sequence in a BAC collection contains a
variation in DNA to be related to a trait, this variation can be hidden/confounded by
other independent and random variations within the same BAC, therefore a single
alignment may not be effective in detecting the targeted variation(s). The first stage
of this process involves an alignment of raw sequences with an open:extension cost
ratio greater than 10. The second stage consists of trimming the tails (noise) and
realigning the original sequence, whose cluster will already indicate the BAC
potential for bearing a region of interest. Subsequent stages consist of upstream or
downstream trimming of random allelic variation, i.e., alleles inside the sequence
that showed diversity across any phenotype. UPGMA (Unweighted Pair Group
Method with Arithmetic Mean) can then be applied to the resulting alignment until a
phenogram identifies a unique cluster exclusive to lines having a desired trait or
phenotype. The final cluster can then be used to identify the specific variation that
will be used for primer design to generate a trait specific marker.
EXAMPLES

The following examples are offered to illustrate, but not to limit, the appended claims. It is understood that the examples and embodiments described herein are for illustrative purposes only and that persons skilled in the art will recognize various reagents or parameters that can be altered without departing from the spirit of the invention or the scope of the appended claims.

EXAMPLE 1

Phenotypic Assessment of Enhanced Resistance to Tropical and Southern Rust

Maize plants were inoculated in the field with *Physopella zeae* to promote symptoms of tropical rust. Three inoculations were done starting at the V10 stage (each inoculation with -500,000 spores per ml to a total volume of 200 liters per hectare).

Maize plants were evaluated for enhanced resistance to southern rust (pathogen *Puccinia polysora*) based on natural infection conditions in a field located in Itumbiara, Brazil.

Tropical rust develops from the top of the plant to the bottom of the plant, while southern rust moves from the bottom of the plant to the top. Thus, in the same plant, it is possible to observe symptoms of both tropical and southern rust diseases.

Two systems were used to score the plants in the field at the R2 stage (around 20 days after pollination time). The first system is on a 1 to 9 scale, where 1 = most susceptible and 9 = most resistant, while the second system corresponds to the Modified International Standard Scale for Rust (Table 4).

<table>
<thead>
<tr>
<th>International Symbol</th>
<th>PiONEER Scale</th>
<th>Description of the host: Parasite interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oi</td>
<td>9</td>
<td>Immune - No signs of infection</td>
</tr>
<tr>
<td>Oc</td>
<td>8</td>
<td>Highly resistant, minute chlorotic flecks</td>
</tr>
<tr>
<td>On</td>
<td>7</td>
<td>Highly resistant, minute necrotic flecks</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>Resistant, small pustules with necrotic surrounding tissue</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>Mod. Resistant, medium size pustules with necrotic surrounding tissue</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>Mod. Susceptible, medium size pustules with chlorotic surrounding tissue</td>
</tr>
</tbody>
</table>
Susceptible. Large pustules with little or no chlorosis

Most Severe Mesotheric reaction. Mixed reaction types on one leaf

4  2  Susceptible. Large pustules with little or no chlorosis
X Most Severe score observed Mesotheric reaction. Mixed reaction types on one leaf

EXAMPLE 2
Development of PHS6Y Inbred population

PHS6Y was developed using a pedigree selection scheme at the Itumbiara Research Center in Brazil. Individual ears were selected in the F2 generation from a cross between PH7W3 and CML339. CML339 (Makumbi et al. Combining Ability and Heterosis in Tropical Maize Inbreds under Stress and Optimal Conditions. The ASA-CSSA-SSSA International Annual Meetings (November 6-10, 2005), Salt Lake City, UT.2005) is a line with enhanced resistance to tropical rust that was obtained from the International Maize and Wheat Improvement center (CIMMYT). Each selected F2 ear was planted as an F3 row in the following generation. Three ears from each selected F3 row were then planted in the next generation, and the best F4 row was selected and the seed designated as PHS6Y inbred seed. All selections were performed based on the tropical rust resistance phenotype.

EXAMPLE 3
Segregation for Tropical Rust Resistance Indicates that a Single Dominant Gene is Responsible for Conferring Tropical Rust Resistance

An F2 population was developed from a cross between PH468, an inbred susceptible to tropical rust, and PHS6Y, the inbred identified in EXAMPLE 2.

Frequency distributions demonstrating the tropical and southern rust scores for individuals in the PH468 x PHS6Y F2 population are shown in FIGs. 2A and 2B, respectively. Each distribution indicates a segregation ratio of 3 resistant (scores ≥ 5 on the 1-9 scale): 1 susceptible (scores < 5 on the 1-9 scale) for tropical rust (FIG. 2A) and for southern rust (FIG. 2B).

Table 5 shows a Chi-square test which provides evidence for the presence of a single dominant gene that confers resistance to tropical rust, wherein the favorable genotype is present in the PHS6Y inbred. Results also show that a single dominant gene confers resistance to southern rust. However, based on genetic recombination frequencies between tropical and southern resistance in two F2 populations in which PHS6Y is a parent, the genes that confer resistance appear to
be different for tropical and southern rust (Table 6).

**TABLE 5**

<table>
<thead>
<tr>
<th>Population PH468/PHS6Y</th>
<th>Tropical rust score</th>
<th># Plants</th>
<th>p-value</th>
<th>CHI-Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed</td>
<td>&lt;5</td>
<td>305</td>
<td>≥5</td>
<td>0.128</td>
</tr>
<tr>
<td>Expected</td>
<td>292</td>
<td>84</td>
<td>97</td>
<td>2.321</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Southern rust score</th>
<th># Plants</th>
<th>p-value</th>
<th>CHI-Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed</td>
<td>298</td>
<td>9</td>
<td>0.482</td>
</tr>
<tr>
<td>Expected</td>
<td>292</td>
<td>91</td>
<td>0.494</td>
</tr>
</tbody>
</table>

Table 6 shows the genetic recombination frequencies between tropical and southern resistance in two F2 populations with PHS6Y. Contr is another F2 population where both tropical and southern rust traits are also segregating but in an independent manner.

**TABLE 6.**

<table>
<thead>
<tr>
<th>Genomic Recombination Between Tropical and Southern Resistance in Two F2 Populations with PHS6Y as a Parent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>F2 POPULATION</td>
</tr>
<tr>
<td># Recomb</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>468/S6Y</td>
</tr>
<tr>
<td>467/S6Y</td>
</tr>
<tr>
<td>Control (7513/26N)</td>
</tr>
</tbody>
</table>

468 = PH468; S6Y = PHS6Y; 467 = PH467

**EXAMPLE 4**

**Composite Interval Mapping**

A composite interval mapping approach that combines interval mapping with linear regression was undertaken to identify maize chromosomal intervals and markers associated with resistance to tropical rust. In an interval mapping approach
(Lander and Botstein, Genetics 121:185-199 (1989)), each of many positions along
the genetic map (say at 1 cM intervals) is tested for the likelihood that a gene or
QTL controlling a trait of interest is located at that position. The
genotype/phenotype data are used to calculate for each test position a LOD score
(log of likelihood ratio). When the LOD score exceeds a threshold value (herein the
threshold value is 2.5), there is significant evidence for the location of a gene or QTL
at that position on the genetic map (which will fall between two particular marker
loci).

Windows QTL Cartographer (the most up-to-date version of this software was
used at the date of QTL mapping) was used to perform the composite interval
mapping. LOD scores (logarithm of the odds ratio) were estimated across the
genome according to standard QTL mapping procedures.

Results from composite interval mapping for resistance to tropical rust using
the PH468 x PHS6Y F2 population are shown in FIG. 3. The composite interval
mapping analysis detected a large effect QTL on chromosome 10 (FIG. 3) located
between markers C00441-801 and C00428-801. The linkage map used for
composite interval mapping was an internally-derived proprietary genetic map
(identified herein as “PHB”) for which the genetic distances correspond to a single
meiosis recombination fraction. Marker positions on the x-axis correspond to the
PHB genetic map. The y-axis represents the LOD score.

EXAMPLE 5

Backcrossing of the Tropical Rust Resistance Locus from PHS6Y into Susceptible
Inbred Lines

The tropical rust resistant inbred line PHS6Y was elected as the donor parent
for the backcrossing program. This inbred line carries a favorable allele within the
segment of Chromosome 10 harboring the tropical rust gene. In the initial
backcrossing program, four inbred lines (PH9VF, PHDGA, PH467 and PHOTJ) were
elected to be converted with the tropical rust resistance locus from PHS6Y.

Each inbred line (PH9VF, PHDGA, PH467 and PHOTJ) was crossed to
PHS6Y. After obtaining F1 seed from each cross, five subsequent backcrosses
were performed in which the susceptible parent was used as the recurrent parent.
Two generations a year were evaluated to accelerate the process, one in North of
Brazil at the Balsas Winter Nursery location and the other at Itumbiara Research Center. In the first backcross, only phenotypic selection was done at Itumbiara center. In subsequent backcrosses, marker-assisted selection was performed. The backcrossing process was followed by two generations of selfing to fix the resistant allele in each inbred.

Three to six markers were used in the process of converting each inbred (Table 7). The markers were used in the Backcross 2 (BC2) generation up to the BC4 generation as well as for the identification of homozygous plants carrying the resistance alleles at BC4F2.

Table 7 shows the markers on Chromosome 10 that were used for introgressing the resistance locus from PHS6Y into inbreds PH9VF, PHDGA, PH467, and PHOTJ, and the polymorphisms between each susceptible inbred and PHS6Y.

Other inbreds were converted to have the tropical rust resistance locus from PHS6Y. The conversions were done similarly as described above except more markers were used for the conversion. Table 8 shows the markers on Chromosome 10 that were used for introgressing the susceptible resistance locus from PHS6Y into 18 inbreds and the polymorphisms between each susceptible inbred and PHS6Y.

**TABLE 7**

<table>
<thead>
<tr>
<th>CHROMOSOME 10 MARKERS USED FOR CONVERSION OF FOUR INBREDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>INBRED</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>PHS6Y</td>
</tr>
<tr>
<td>PH9VF</td>
</tr>
</tbody>
</table>

47
1 = "A", 2 = "C", 3 = "G", 4 = "T", 5 = "I" or insertion, 6 = "D" or deletion

Table 8
Chromosome 10 Markers Used for Conversion of Eighteen Inbreds

<table>
<thead>
<tr>
<th>Marker</th>
<th>PHM 1192-</th>
<th>PHM 1192-</th>
<th>PHM13818-</th>
<th>PHM 15721-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C00071-01</td>
<td>26</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>PH6S6Y</td>
<td>6,6</td>
<td>1,1</td>
<td>5,5</td>
<td>2,2</td>
</tr>
<tr>
<td>PH92E</td>
<td>6,6</td>
<td>1,1</td>
<td>6,6</td>
<td>2,2</td>
</tr>
<tr>
<td>PH0R8</td>
<td>6,6</td>
<td>3,3</td>
<td>n/a</td>
<td>2,2</td>
</tr>
<tr>
<td>PHOTJ</td>
<td>6,6</td>
<td>3,3</td>
<td>5,5</td>
<td>2,2</td>
</tr>
<tr>
<td>PH1BC</td>
<td>6,6</td>
<td>1,1</td>
<td>6,6</td>
<td>2,2</td>
</tr>
<tr>
<td>PH56SM</td>
<td>6,6</td>
<td>3,3</td>
<td>5,5</td>
<td>2,2</td>
</tr>
<tr>
<td>PHKTE</td>
<td>6,6</td>
<td>3,3</td>
<td>n/a</td>
<td>2,2</td>
</tr>
<tr>
<td>PH57S</td>
<td>5,5</td>
<td>1,1</td>
<td>5,5</td>
<td>2,2</td>
</tr>
<tr>
<td>PH97TJ</td>
<td>5,5</td>
<td>1,1</td>
<td>5,5</td>
<td>2,2</td>
</tr>
<tr>
<td>PH5BNF</td>
<td>5,5</td>
<td>1,1</td>
<td>5,5</td>
<td>2,2</td>
</tr>
<tr>
<td>PH5D18</td>
<td>5,5</td>
<td>1,1</td>
<td>5,5</td>
<td>2,2</td>
</tr>
<tr>
<td>PHR33</td>
<td>6,6</td>
<td>3,3</td>
<td>5,5</td>
<td>2,2</td>
</tr>
<tr>
<td>PH59VC</td>
<td>6,6</td>
<td>1,1</td>
<td>6,6</td>
<td>2,2</td>
</tr>
<tr>
<td>PH5KNC</td>
<td>5,5</td>
<td>1,1</td>
<td>n/a</td>
<td>2,2</td>
</tr>
<tr>
<td>PH819</td>
<td>6,6</td>
<td>1,1</td>
<td>6,6</td>
<td>2,2</td>
</tr>
<tr>
<td>PH5KNF</td>
<td>6,6</td>
<td>1,1</td>
<td>5,5</td>
<td>2,2</td>
</tr>
<tr>
<td>PH59V7</td>
<td>5,5</td>
<td>1,1</td>
<td>5,5</td>
<td>2,2</td>
</tr>
<tr>
<td>PH5DNN</td>
<td>5,5</td>
<td>1,1</td>
<td>5,5</td>
<td>2,2</td>
</tr>
<tr>
<td>PH5M3M</td>
<td>5,5</td>
<td>1,1</td>
<td>5,5</td>
<td>2,2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>PHM 15721-</th>
<th>PHM 18427-</th>
<th>PHM4003-</th>
<th>PHM4370-19</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH56Y</td>
<td>6,6</td>
<td>6,6</td>
<td>1,1</td>
<td>2,2</td>
</tr>
<tr>
<td>PH592E</td>
<td>n/a</td>
<td>5,5</td>
<td>1,1</td>
<td>2,2</td>
</tr>
<tr>
<td>PH50R8</td>
<td>n/a</td>
<td>6,6</td>
<td>n/a</td>
<td>2,2</td>
</tr>
<tr>
<td>PH50TJ</td>
<td>6,6</td>
<td>6,6</td>
<td>3,3</td>
<td>2,2</td>
</tr>
<tr>
<td>PH51BC</td>
<td>6,6</td>
<td>5,5</td>
<td>1,1</td>
<td>2,2</td>
</tr>
<tr>
<td>PH556SM</td>
<td>n/a</td>
<td>n/a</td>
<td>3,3</td>
<td>2,2</td>
</tr>
<tr>
<td>PH5KTE</td>
<td>6,6</td>
<td>6,6</td>
<td>3,3</td>
<td>2,2</td>
</tr>
<tr>
<td>PH57S</td>
<td>5,5</td>
<td>6,6</td>
<td>1,1</td>
<td>2,2</td>
</tr>
<tr>
<td>PH59TJ</td>
<td>6,6</td>
<td>6,6</td>
<td>1,1</td>
<td>2,4</td>
</tr>
<tr>
<td>PH55BNF</td>
<td>6,6</td>
<td>6,6</td>
<td>1,1</td>
<td>2,2</td>
</tr>
<tr>
<td></td>
<td>PHM3M</td>
<td>PHM5028-6</td>
<td>PHM9535-2</td>
<td>PHM9535-6</td>
</tr>
<tr>
<td>-------</td>
<td>-------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>PHD18</td>
<td>6,6</td>
<td>6,6</td>
<td>1,1</td>
<td>2,2</td>
</tr>
<tr>
<td>PHR33</td>
<td>6,6</td>
<td>6,6</td>
<td>3,3</td>
<td>2,2</td>
</tr>
<tr>
<td>PH9VC</td>
<td>5,5</td>
<td>5,5</td>
<td>1,1</td>
<td>2,2</td>
</tr>
<tr>
<td>PH9VC</td>
<td>5,5</td>
<td>6,6</td>
<td>1,1</td>
<td>2,2</td>
</tr>
<tr>
<td>PH9VC</td>
<td>5,5</td>
<td>5,5</td>
<td>1,1</td>
<td>2,2</td>
</tr>
<tr>
<td>PH9VC</td>
<td>5,5</td>
<td>6,6</td>
<td>1,1</td>
<td>2,2</td>
</tr>
<tr>
<td>PH9VC</td>
<td>6,6</td>
<td>6,6</td>
<td>1,1</td>
<td>n/a</td>
</tr>
<tr>
<td>PH9VC</td>
<td>6,6</td>
<td>6,6</td>
<td>1,1</td>
<td>2,2</td>
</tr>
<tr>
<td>PH9VC</td>
<td>6,6</td>
<td>6,6</td>
<td>n/a</td>
<td>4,4</td>
</tr>
<tr>
<td>PHM5028-6</td>
<td>24</td>
<td>107</td>
<td>10</td>
<td>PHM9535-6</td>
</tr>
<tr>
<td>PHS6Y</td>
<td>4,4</td>
<td>2,2</td>
<td>3,3</td>
<td>1,1</td>
</tr>
<tr>
<td>PH92E</td>
<td>4,4</td>
<td>2,2</td>
<td>3,3</td>
<td>1,1</td>
</tr>
<tr>
<td>PH0R8</td>
<td>2,2</td>
<td>4,4</td>
<td>3,3</td>
<td>1,1</td>
</tr>
<tr>
<td>PH0R8</td>
<td>2,2</td>
<td>4,4</td>
<td>3,3</td>
<td>1,1</td>
</tr>
<tr>
<td>PH1BC</td>
<td>4,4</td>
<td>n/a</td>
<td>4,4</td>
<td>4,4</td>
</tr>
<tr>
<td>PH57S</td>
<td>4,4</td>
<td>n/a</td>
<td>4,4</td>
<td>4,4</td>
</tr>
<tr>
<td>PH97TJ</td>
<td>4,4</td>
<td>2,2</td>
<td>3,3</td>
<td>4,4</td>
</tr>
<tr>
<td>PHBNF</td>
<td>4,4</td>
<td>4,4</td>
<td>4,4</td>
<td>4,4</td>
</tr>
<tr>
<td>PHD18</td>
<td>4,4</td>
<td>4,4</td>
<td>3,3</td>
<td>1,1</td>
</tr>
<tr>
<td>PHR33</td>
<td>2,2</td>
<td>4,4</td>
<td>3,3</td>
<td>1,1</td>
</tr>
<tr>
<td>PH9VC</td>
<td>2,2</td>
<td>4,4</td>
<td>4,4</td>
<td>1,1</td>
</tr>
<tr>
<td>PH9VC</td>
<td>2,2</td>
<td>4,4</td>
<td>3,3</td>
<td>1,1</td>
</tr>
<tr>
<td>PH9VC</td>
<td>2,2</td>
<td>2,2</td>
<td>4,4</td>
<td>1,1</td>
</tr>
<tr>
<td>PH9VC</td>
<td>2,2</td>
<td>n/a</td>
<td>3,3</td>
<td>1,1</td>
</tr>
<tr>
<td>PH9VC</td>
<td>2,2</td>
<td>n/a</td>
<td>3,3</td>
<td>4,4</td>
</tr>
<tr>
<td>PHM3M</td>
<td>4,4</td>
<td>n/a</td>
<td>3,3</td>
<td>4,4</td>
</tr>
<tr>
<td>PHM3M</td>
<td>4,4</td>
<td>n/a</td>
<td>3,3</td>
<td>4,4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>C00435-802-U</th>
<th>C00436-801</th>
<th>C00423-801</th>
<th>C00441-801</th>
<th>C00441-802</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHS6Y</td>
<td>4,4</td>
<td>3,3</td>
<td>4,4</td>
<td>3,3</td>
<td>4,4</td>
</tr>
<tr>
<td>PH92E</td>
<td>1,1</td>
<td>n/a</td>
<td>2,2</td>
<td>4,4</td>
<td>n/a</td>
</tr>
<tr>
<td>PH0R8</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>PH0R8</td>
<td>1,1</td>
<td>1,1</td>
<td>2,2</td>
<td>4,4</td>
<td>2,2</td>
</tr>
<tr>
<td>PH0R8</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>PH1BC</td>
<td>1,1</td>
<td>3,3</td>
<td>n/a</td>
<td>4,4</td>
<td>n/a</td>
</tr>
<tr>
<td>PH57S</td>
<td>1,1</td>
<td>1,1</td>
<td>2,2</td>
<td>4,4</td>
<td>2,2</td>
</tr>
<tr>
<td>PH57S</td>
<td>n/a</td>
<td>1,1</td>
<td>n/a</td>
<td>4,4</td>
<td>2,2</td>
</tr>
<tr>
<td>PH57S</td>
<td>4,4</td>
<td>3,3</td>
<td>4,4</td>
<td>3,3</td>
<td>4,4</td>
</tr>
<tr>
<td>PH57S</td>
<td>4,4</td>
<td>3,3</td>
<td>2,2</td>
<td>4,4</td>
<td>2,2</td>
</tr>
<tr>
<td>PH57S</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>PH57S</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>PH57S</td>
<td>1,1</td>
<td>1,1</td>
<td>2,2</td>
<td>4,4</td>
<td>2,2</td>
</tr>
</tbody>
</table>

49
<table>
<thead>
<tr>
<th>Variety</th>
<th>Conversion</th>
<th>Field</th>
<th>Disease Resistance</th>
<th>Hybrid Production with Enhanced Resistance to Tropical Rust</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH9VC</td>
<td>1.1</td>
<td>1.1</td>
<td>n/a</td>
<td>The converted inbreds were used to make hybrids, and the field trial results have shown that the excellent level of resistance seen in the converted inbreds (for example, FIG. 4) is maintained in hybrids made with the conversion inbreds (FIG. 5; e.g. hybrid GEID61 70295).</td>
</tr>
<tr>
<td>PHKNC</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>PH819</td>
<td>1.1</td>
<td>3.3</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>PHKNF</td>
<td>4.4</td>
<td>n/a</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>PH9V7</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>PHDNV</td>
<td>11</td>
<td>n/a</td>
<td>2,2</td>
<td></td>
</tr>
<tr>
<td>PHM3M</td>
<td>n/a</td>
<td>1,1</td>
<td>n/a</td>
<td></td>
</tr>
</tbody>
</table>

C00428-801

PHS6Y 1,1

PH92E 1,1

PH0R8 3,3

PHOTJ 3,3

PH1BC 1,1

PHS6M 1,1

PHKTE 3,3

PHS7S 3,3

PH9TJ 1,1

PHBENF n/a

PHD18 n/a

PHR33 1 3,3

PH9VC 3,3

PHKNF n/a

PHH19 3,3

PH9V7 n/a

PHDNV 1,1

PHM3M 1,1

1 = "A", 2 = "C", 3 = "G", 4 = "T", 5 = 1 " or insertion, 6 = "D" or deletion

5

EXAMPLE 6

Hybrid Production with Enhanced Resistance to Tropical Rust

The converted inbreds were used to make hybrids, and the field trial results have shown that the excellent level of resistance seen in the converted inbreds (for example, FIG. 4) is maintained in hybrids made with the conversion inbreds (FIG. 5; e.g. hybrid GEID61 70295).
EXAMPLE 7
Genotyping of Maize Lines for Tropical Rust Resistance and Identification of Polymorphisms Associated with Enhanced Tropical Rust Resistance

Genotyping of maize lines for tropical rust resistance

Resistant and susceptible lines were genotyped by Sanger re-sequencing of genomic targets. The targets were PCR-amplified products from single-copy genomic sections of the tropical rust QTL region mapped on the short arm of chromosome 10.

Available public genomic sequence was used as reference for primer design.

The public sequence corresponds to inbred line B73 and was obtained by a BAC minimum tiling path strategy (available on the Maize Genome Browser, which is publicly available on the internet). The following sequenced BAC clones have been mapped to the region of interest: c0497H2, c0284b01, c0446H0, c0340m14, c0178k23, c0332e10, c0009k11, c0281e11, c0230k24, b0286c12, c0044b04, c0149n21, c0064g11, c0118o03, b0191e02, c0462j05. While the order and orientation of the BACs in the tiling path has been determined by fingerprinting (Nelson et al., 2005. Whole-Genome Validation of High-Information-Content Fingerprinting. Plant Physiology. 139:27-38), the order and orientation of sequence contigs within each clone has not been fully determined. For this work, internal information on BAC overlap was used to further narrow the sequence order of the 2-2.5 Mb region into 24 sub-regions (FIG. 6).

The sequence includes large portions of highly repetitive DNA, mostly as retrotransposon-like sequences. Multiple-copy sequence tracks were identified and removed from the sequence by masking repeats using Cross-match (http://www.phrap.org). Low copy sequences were further identified and removed by BLAST analysis.

PCR primers were initially designed to amplify 270 to 720-bp amplicons in single-copy tracts spanning the 24 sub-regions in the chromosome 10 target region (Table 9). Primers sets were designed using proprietary tools based on Primer3 (Rozen, S. and Skaletsky, 2000. Primer3 on the WWW for general users and for biologist programmers, Methods Mol Biol. 132:365-386.). Sequencing primers M13R (5-GGAAACAGCTATGACCATG) and M13F (5-TGTAATACGACGCTTATTG)
were added to the L and R primers, respectively, as tails to facilitate sequencing. Quality and uniqueness of PCR assays were validated by performing and analyzing preliminary PCR and sequencing on control DNA samples from lines B73 and Mo17. Maize-oat addition line amplification was used to further validate assays. PCR primers that produced amplified products in multiple addition lines or did not produce an amplified product exclusively in the chromosome 10 maize-oat addition line were discarded.

**TABLE 9**

<table>
<thead>
<tr>
<th>Marker Name</th>
<th>Sub-region</th>
<th>B73 BAC</th>
<th>Clone ID</th>
<th>L primer (no M13 tail)</th>
<th>R primer (no M13 tail)</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub2e</td>
<td>2</td>
<td>c0497L12</td>
<td>Ct9050c497L12e</td>
<td>SEQ ID NO: 129</td>
<td>SEQ ID NO: 130</td>
<td>616</td>
</tr>
<tr>
<td>Sub19d</td>
<td>19</td>
<td>c0064G11</td>
<td>Ct9050c064G11d</td>
<td>SEQ ID NO: 131</td>
<td>SEQ ID NO: 132</td>
<td>601</td>
</tr>
<tr>
<td>Sub19c</td>
<td>19</td>
<td>c0064G11</td>
<td>Ct9050c064G11c</td>
<td>SEQ ID NO: 133</td>
<td>SEQ ID NO: 134</td>
<td>649</td>
</tr>
<tr>
<td>Sub23m</td>
<td>23</td>
<td>c0462J05</td>
<td>Ct9050b191E02m</td>
<td>SEQ ID NO: 135</td>
<td>SEQ ID NO: 136</td>
<td>605</td>
</tr>
</tbody>
</table>

PCR was performed on 10-30 ng DNA using HotStar Taq Polymerase Master Mix (Qiagen), according to recommendations by the manufacturers with some modifications. The total reaction volume was 10 µl and contained 5U HotStar Taq DNA polymerase, 1.5mM MgCl2, 200µM dNTPs and 5pM of each tailed primer. PCR amplification was performed as follows: 1) 15-minute initial step at 95°C; 2) 40 cycles of 30 seconds at 95°C, 30 seconds at 60°C, 1 minute at 72°C; and 3) final extension step of 10 minutes at 72°C. PCR products were confirmed by gel electrophoresis. One fifth (2 µl) of the PCR reaction was diluted in 17 µl of sterile distilled water and cleaned up with 0.5 to 0.75 µl ExoSAP-IT (USB Corporation), incubating at 37°C for 25min then 80°C for 25min.

Bidirectional cycle sequencing of PCR amplicons was performed using Big Dye Terminator cycle sequencing protocols and capillary sequencing in Applied Biosystems 3730 XL DNA analyzers. 3-5 µl of the cleaned-up DNA was sequenced using M13F and M13R oligonucleotides and the BigDye Prism sequencing kit (ABI;
version 3.1), according to manufacturer conditions. After cycle sequencing, reaction products were cleaned up by ethanol precipitation and processed on ABI3730xl automated sequencers (ABI), according to standard protocols.


Identification of polymorphisms associated with enhanced resistance to tropical rust

DNA fragments bearing a high level of internal diversity are more likely to contain genes of interest. However, designing primers in these regions can be difficult because primer design applications have a tendency to select areas of random variation for primer design. A multiple stages cluster methodology was tested in order to direct primer design into non-random variation. This method uses phylogenetic trees and a sequential alignment process to identify unique regions containing allelic variations exclusive to lines with enhanced resistance to tropical rust. The first stage of this process involves an alignment of raw sequences with an open:extension cost ratio greater than 10. The second stage consists of trimming the tails (noise). Subsequent stages consist of trimming random allelic variation, i.e., alleles inside the sequence that showed diversity across any phenotype. UPGMA (Unweighted Pair Group Method with Arithmetic Mean) is then applied to the resulting alignment until a phenogram identifies a unique cluster exclusive to resistant lines.

One primer pair (SEQ ID NO:133 and SEQ ID NO:134) produced an amplicon with the reference sequence (SEQ ID NO:155) that is referred to herein as PHMTR. All lines that showed resistance to tropical rust contained a T-deletion of bp 16 of PHMTR (the sequence of the PHMTR-T region is SEQ ID NO:156) while all
maize lines susceptible to tropical rust contained an intact PHMTR region (SEQ ID NO: 155).

FIG. 7 shows a part of the reference sequence (top) obtained by the genotyping of maize lines resistant and susceptible to tropical rust using PCR primers (SEQ ID NO: 133 and 134) designed for clone ID Ct9050c064G1 1c (Table 9). SEQ ID NOs:137-142 represent amplicons obtained from resistant lines, while SEQ ID NOs:143-1 54 represent amplicons obtained from susceptible lines. The area highlighted in grey represents a 21 bp-region of the reference sequence (SEQ ID NO:155).

Amplicon sequences were also obtained using primers SEQ ID NO:135 and SEQ ID NO:136 (SEQ ID NO:167 is the reference sequence for this region) and eight independent clusters. Table 10 shows 21 lines evaluated for the cluster analysis. A GAG haplotype (at positions 337-339 of reference sequence SEQ ID NO:167; see FIG. 8) was found to be unique to all lines with enhanced resistance to tropical rust (Table 10 and FIG. 8). Two new presence/absence markers were developed to assay this haplotype, C06621-1-K2 and C06621-1-K4 (Table 3; SEQ ID NOs: 157-164), using the KASPar assay techniques described on the kbioscience website. C06621-1-K2 and C06621-1-K4 are X/P type markers, where X indicates absence and P indicates presence. The P marker detects the GAG polymorphism and the X marker detects ADH, an internal control gene, which is used to show that the reaction worked. Eighteen lines were evaluated with the C06621-1-K2 and C06621-1-K4 markers. All fit the expectation for both markers (except one sample that had missing data) given their cluster analysis.

**TABLE 10**

<table>
<thead>
<tr>
<th>Group</th>
<th>Line</th>
<th>Haplotype</th>
<th>Trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>PHS6Y</td>
<td>GAG</td>
<td>Tropical and Southern rust</td>
</tr>
<tr>
<td>A</td>
<td>PH1 FT71</td>
<td>GAG</td>
<td>Tropical and Southern rust</td>
</tr>
<tr>
<td>A</td>
<td>PH1JG22</td>
<td>GAG</td>
<td>Tropical and Southern rust</td>
</tr>
<tr>
<td>A</td>
<td>PH1G3H1</td>
<td>GAG</td>
<td>Tropical and Southern rust</td>
</tr>
<tr>
<td>A</td>
<td>PH1JG01</td>
<td>GAG</td>
<td>Tropical and Southern rust</td>
</tr>
<tr>
<td>B</td>
<td>PH9VF</td>
<td>ACA</td>
<td>None</td>
</tr>
<tr>
<td>C</td>
<td>A63</td>
<td>ACG</td>
<td>None</td>
</tr>
<tr>
<td>C</td>
<td>PH9PR</td>
<td>ACG</td>
<td>None</td>
</tr>
<tr>
<td>C</td>
<td>PH7WC</td>
<td>ACG</td>
<td>None</td>
</tr>
<tr>
<td>C</td>
<td>PH48F</td>
<td>ACG</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Marker</td>
<td>Type</td>
<td>Description</td>
</tr>
<tr>
<td>---</td>
<td>----------</td>
<td>--------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>C</td>
<td>PHDGA</td>
<td>ACG</td>
<td>None</td>
</tr>
<tr>
<td>C</td>
<td>A63-1</td>
<td>ACG</td>
<td>None</td>
</tr>
<tr>
<td>C</td>
<td>PH7W3</td>
<td>ACG</td>
<td>None</td>
</tr>
<tr>
<td>C</td>
<td>PHOTJ</td>
<td>ACG</td>
<td>None</td>
</tr>
<tr>
<td>D</td>
<td>PHBNA</td>
<td>GCG</td>
<td>None</td>
</tr>
<tr>
<td>D</td>
<td>Mo17</td>
<td>GCG</td>
<td>Common rust</td>
</tr>
<tr>
<td>D</td>
<td>PH467</td>
<td>GCG</td>
<td>None</td>
</tr>
<tr>
<td>D</td>
<td>PH147G5</td>
<td>GCG</td>
<td>Common rust</td>
</tr>
<tr>
<td>D</td>
<td>PHP3P1</td>
<td>GCG</td>
<td>Common rust</td>
</tr>
<tr>
<td>D</td>
<td>PH1AGK1</td>
<td>GCG</td>
<td>Common rust</td>
</tr>
<tr>
<td>D</td>
<td>PHY7M2</td>
<td>GCG</td>
<td>Common rust</td>
</tr>
</tbody>
</table>

The multiple stages cluster methodology proved to be an efficient method to identify unique regions containing allelic variations exclusive to lines with enhanced resistance to tropical rust, and this methodology can be applied to any trait of interest.

**EXAMPLE 8**

Markers and/or Haplotypes for Use in Marker Assisted Selection of Maize Plants with Enhanced Resistance to Tropical Rust

A set of common markers can be used to aid in the identification of other markers that can be used to select for maize plants with enhanced resistance to tropical rust. Table 11 shows markers identified herein that define the interval comprising a gene that confers resistance to tropical rust. Markers are in physical map order (as depicted in FIG. 1). The positions of the markers on the PHB internally derived map (based on single meiosis) and on the IBM2 neighbors genetic map (high resolution B73/Mo17 genetic map) are also shown.
Closely linked markers flanking the locus of interest that have alleles in linkage disequilibrium with a resistance allele at that locus may be effectively used to select for progeny plants with enhanced resistance to tropical rust. Thus, the markers described herein, such as those listed in Table 11, as well as other markers genetically or physically mapped to the same chromosomal segment, may be used to select for maize plants with enhanced resistance to tropical rust. Typically, a set of these markers will be used, (e.g., 2 or more, 3 or more, 4 or more, 5 or more) in the flanking region above the gene and a similar set in the flanking region below the gene. Optionally, a marker within the actual gene and/or locus may also be used.

### Table 11
Molecular Marker Positions on the PHB map and the IBM2 Neighbors map

<table>
<thead>
<tr>
<th>Marker Locus</th>
<th>PHB map position (cM)</th>
<th>IBM2 neighbors</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHM15590</td>
<td>11.7</td>
<td>na</td>
</tr>
<tr>
<td>C00441-801</td>
<td>13.0</td>
<td>na</td>
</tr>
<tr>
<td>C00441-802</td>
<td>13.6</td>
<td>na</td>
</tr>
<tr>
<td>PHM13818-15</td>
<td>10.8</td>
<td>na</td>
</tr>
<tr>
<td>PHM1192-26</td>
<td>4.1</td>
<td>na</td>
</tr>
<tr>
<td>PHM1192-4</td>
<td>4.1</td>
<td>na</td>
</tr>
<tr>
<td>PHM187-7</td>
<td>9.3</td>
<td>na</td>
</tr>
<tr>
<td>C00435-802</td>
<td>6.3</td>
<td>na</td>
</tr>
<tr>
<td>C00436-801</td>
<td>7.8</td>
<td>na</td>
</tr>
<tr>
<td>PHM5028-24</td>
<td>9.7</td>
<td>na</td>
</tr>
<tr>
<td>C00423-801</td>
<td>9.4</td>
<td>na</td>
</tr>
<tr>
<td>PHM4370-19</td>
<td>15.0</td>
<td>na</td>
</tr>
<tr>
<td>C00071-01</td>
<td>18.2</td>
<td>na</td>
</tr>
<tr>
<td>C00428-801</td>
<td>20.2</td>
<td>na</td>
</tr>
<tr>
<td>PHM731-107</td>
<td>18.2</td>
<td>19.1</td>
</tr>
<tr>
<td>PHM15721-39</td>
<td>12.6</td>
<td>na</td>
</tr>
<tr>
<td>PHM15721-180</td>
<td>12.6</td>
<td>na</td>
</tr>
<tr>
<td>PHM8249-21</td>
<td>19.5</td>
<td>na</td>
</tr>
<tr>
<td>PHM18427-13</td>
<td>20.4</td>
<td>na</td>
</tr>
<tr>
<td>PHM4003-13</td>
<td>25.4</td>
<td>na</td>
</tr>
<tr>
<td>PHM9535-10</td>
<td>24.2</td>
<td>29.6</td>
</tr>
<tr>
<td>PHM9535-6</td>
<td>24.2</td>
<td>29.6</td>
</tr>
<tr>
<td>PHM9535-7</td>
<td>24.2</td>
<td>29.6</td>
</tr>
</tbody>
</table>

na = not available
The parents and their progeny are screened for these sets of markers, and the markers that are polymorphic between the two parents are used for selection. The most proximal polymorphic markers to the gene or locus are used to select for the gene or locus, and the more distal polymorphic markers are used to select against the gene or locus. In an introgression program, this allows for selection of the gene or locus genotype at the more proximal polymorphic markers and selection for the recurrent parent genotype at the more distal polymorphic markers.

A haplotype, or a combination of alleles, can also be used to select for plants in a breeding program. Haplotypes can be more informative than single polymorphisms and can be more descriptive of any particular genotype. Once a unique haplotype has been assigned to a donor chromosomal region, such as a haplotype for PHS6Y in the short arm of chromosome 10, that haplotype can be used in that population or any subset thereof to determine whether an individual has a particular gene. Using automated high throughput marker detection platforms known to those of ordinary skill in the art makes this process highly efficient and effective. The marker alleles disclosed herein can be used alone or in combination to select for plants with enhanced resistance to tropical rust through the use of marker assisted selection.
CLAIMS

What is claimed:

1. A method of selecting a maize plant with enhanced resistance to tropical rust comprising:
   a. detecting in the maize plant a first marker allele that is linked to and associated with:
      i. a "T" deletion at position 16 of PHMTR; or
      ii. a "GAG" haplotype at positions 337-339 of reference sequence SEQ ID NO:167; and
   b. selecting said maize plant that has the first marker allele.

2. The method of claim 1, wherein the first marker allele is linked to the "T" deletion at position 16 of PHMTR or the "GAG" haplotype at positions 337-339 of reference sequence SEQ ID NO:167 by 20 cM on a single meiosis map.

3. The method of claim 1, wherein the first marker allele is linked to the "T" deletion at position 16 of PHMTR or the "GAG" haplotype at positions 337-339 of reference sequence SEQ ID NO:167 by 2 cM on a single meiosis map.

4. A method of selecting a maize plant with enhanced resistance to tropical rust comprising:
   a. detecting in the maize plant
      i. a "T" deletion at position 16 of PHMTR or
      ii. a "GAG" haplotype at positions 337-339 of reference sequence SEQ ID NO:167; and
   b. selecting said maize plant that has the "T" deletion at position 16 of PHMTR or the "GAG" haplotype at positions 337-339 of reference sequence SEQ ID NO:167.

5. A method of identifying a maize plant that displays enhanced resistance to tropical rust, the method comprising detecting in a maize plant a marker locus wherein:
   a. a marker probe comprising all or a portion of the marker locus, or complement thereof, hybridizes under stringent conditions to the contiguous DNA between and including SEQ ID NO:89, or a nucleotide sequence that is 95% identical to SEQ ID NO:89 based on
the Clustal V method of alignment, and SEQ ID NO:96, or a nucleotide sequence that is 95% identical to SEQ ID NO:96 based on the Clustal V method of alignment; and
b. said marker locus comprises at least one allele that is associated with the enhanced resistance to tropical rust.

6. A method of identifying a maize plant that displays enhanced resistance to tropical rust, the method comprising detecting in the germplasm of the maize plant an allele of a marker locus wherein:
   a. the marker locus is located within a chromosomal interval comprising and flanked by PHM15590 and PHM9535; and
   b. the allele is associated with enhanced resistance to tropical rust.

7. The method of claim 6, wherein the marker locus is located within a chromosomal interval comprising and flanked by PHM15590 and PHM15721.

8. The method of claim 6, wherein the marker locus is located within a chromosomal interval comprising and flanked by C0041 and C00428.

9. A method of identifying a maize plant that displays enhanced resistance to tropical rust, the method comprising detecting in the germplasm of the maize plant a haplotype comprising alleles at one or more marker loci, wherein:
   a. the one or more marker loci are located within a chromosomal interval comprising and flanked by PHM15590 and PHM9535; and
   b. the haplotype is associated with enhanced resistance to tropical rust.

10. The method of claim 9, wherein said one or more marker loci are located within a chromosomal interval comprising and flanked by PHM15590 and PHM15721.

11. The method of claim 9 wherein said one or more marker loci are located within a chromosomal interval comprising and flanked by C0041 and C00428.

12. The method of claim 9, 10, or 11, wherein said haplotype comprises a "T" deletion at position 1 of PHMTR or a "GAG" at positions 337-339 of reference sequence SEQ ID NO:167.

13. A method of selecting a maize plant that displays enhanced resistance to, the method comprising:
   a. obtaining a first maize plant having at least one allele of a marker locus, wherein the marker locus is located within a chromosomal
interval comprising and flanked by PHM15590 and PHM9535 and the allele is associated with enhanced resistance to tropical rust; 
b. crossing said first maize plant to a second maize plant; 
c. evaluating progeny plants for the allele of the first maize plant; and 
d. selecting progeny plants that possess the allele of the first maize plant.

14. The method of claim 13, wherein the marker locus is located within a chromosomal interval comprising and flanked by PHM15590 and PHM15721.

15. The method of claim 13, wherein the marker locus is located within a chromosomal interval comprising and flanked by C0041 and C00428.

16. A method of selecting a maize plant that displays enhanced resistance to tropical rust, the method comprising:
   a. obtaining a first maize plant that comprises within its genome:
      i. a "T" deletion at position 16 of PHMTR; or
      ii. a haplotype comprising a "GAG" at positions 337-339 of reference sequence SEQ ID NO:167;
   b. crossing said first maize plant to a second maize plant; 
   c. evaluating progeny plants for the "T" deletion at position 16 of PHMTR or the haplotype comprising a "GAG" at positions 337-339 of reference sequence SEQ ID NO:167; and 
   d. selecting progeny plants that possess the "T" deletion at position 16 of PHMTR or the haplotype comprising a "GAG" at positions 337-339 of reference sequence SEQ ID NO:167.

17. A maize plant identified by any of the methods of claims 5 and 6-12, wherein the plant is not CML339.

18. A maize plant selected by any of the methods of claims 1-4 and 13-16, wherein the plant is not CML339.

19. A method to identify one or more allelic variations associated with a desirable form of a trait comprising:
   a. aligning raw sequences with an open:extension cost ratio greater than 10; 
   b. trimming sequencing tails (noise); 
   c. trimming random allelic variation;
d. applying UPGMA (Unweighted Pair Group Method with Arithmetic Mean) to the resulting alignment;

e. repeating steps c-d until a phenogram identifies a unique cluster of lines with the desirable form of the trait; and

f. identifying the one or more allelic variations associated with the desirable form of a trait.
FIG. 2

PH468 x PHS6Y F2 POPULATION DISTRIBUTION

Frequency

SOURST Score

TRRST Score

Frequency