Validation of fusarium head blight markers in *Triticum aestivum* breeding populations

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ABSTRACT

With quantitative disease resistance, it is difficult to know which loci of a resistant source are passed to progeny that are subsequently used as parents in further crossing. The objective of this study was to identify the Sumai 3 loci that contribute fusarium head blight (FHB) resistance in breeding populations which have Sumai 3 derived parents. Doubled haploid populations of Infinity/ND3085, Infinity/ND744, and Alsen/Helios crosses were evaluated in FHB nurseries at Carman MB, Ottawa ON and Charlottetown PE in 2007 using a two replicate randomized complete block design of 80 lines per population plus parental checks. Percent incidence and severity of head blight, *Fusarium*-damaged kernels and DON accumulation were evaluated. DNA markers reported as associated with Sumai 3 FHB resistance at different loci were assessed on the parents and lines of each population. Statistical analyses were applied to marker and disease results to determine which loci contributed resistance. Resistance was expressed inconsistently across environments and across parents for particular loci. The results demonstrate the need to validate FHB resistance loci from Sumai 3 to determine which were passed to progeny that are subsequently used as parents (eg. Alsen) to efficiently apply marker assisted selection.

INTRODUCTION

Several sources with a high level of resistance to fusarium head blight have been identified in cultivars from around the world[1,2,3]. Sumai 3, a cultivar from China, is a popular source of FHB resistance in other parts of the world[4,5] that is transferred when crossed to locally adapted cultivars[1,7,8]. The transfer of Sumai 3 FHB resistance is challenging because it is controlled by several genes and displays quantitative inheritance[9,10,11]. When multiple genetic loci are involved in resistance, it is possible that the genes will be broken up and not all recombined in progeny with a desirable adapted background. Progeny with improved FHB resistance are subsequently used as parents for further crosses. The only way to know which loci of a resistant source have been passed onto progeny is to test for the presence of resistance alleles at contributing loci. Without the knowledge of which loci are conferring resistance, the efficiency of marker use is reduced because the application of markers at non-contributing loci will be wasted resources. The objective of this study was to determine which Sumai 3 loci contribute FHB resistance

in breeding populations which have Sumai 3 derived parents.

MATERIALS AND METHODS

Doubled haploid[12] spring wheat populations were developed from the crosses Infinity/ND3085, Infinity/ND744, and Alsen/Helios. ND3085[6], ND744[7] and Alsen[8] possess moderate resistance to FHB and all have Sumai 3[4] in their background. Infinity[13] was considered susceptible and Helios[14] intermediate resistant to FHB. The three populations were evaluated in FHB nurseries at Carman MB (two tests), Ottawa ON and Charlottetown PE in 2007. Each test was a two replicate randomized complete block design of 80 lines per population plus three sets of each parental check. Three Infinity plots were added to the Alsen/Helios test to allow cross references between the three trials of populations. The trials were planted as single row plots of 50 seeds per plot. Additional moisture was applied through the period of spike emergence and seed development[15]. Depending on the nursery, either inoculated corn seed was applied near the time of spike emergence[16] or inoculum was sprayed onto spikes[15]. Inoculum was prepared in the laboratory.

Percent incidence and severity of head blight, and *Fusarium*-damaged kernels were evaluated on each replicate. Stratification for anthesis was achieved by rating each line for percent incidence and severity 21 d after anthesis. Percent incidence was calculated as the proportion of spikes with disease times 100. Severity was estimated as the proportion of disease affected spike tissue[17]. Disease index was calculated from the incidence and severity. *Fusarium*-damaged kernels were determined from 50 g threshed grain samples[15]. Deoxynivalinol (DON) accumulation was evaluated on a single replicate using an ELISA test[18].

DNA was extracted from all lines and parents[19]. Microsatellite DNA markers reported as associated with Sumai 3 FHB resistance were assessed on the parents and lines of each population[15,20-28]. Additional markers proximal to published markers were selected based on map information[28,29]. The markers evaluated were gwm349[27,29] (2DL), gwm493[20-22,24-27] (3BS), gwm285[25,27] (3BSc), gwm533.2[20-22,24-27] (3BS), gwm566[27,29] (3BSc), STS3B-66[20,26] (3BS), wmc418[25] (3BSc), wmc471[29] (3BS), wmc505a[27] (3B), wmc653[29] (3BS), wmc48[25,27] (4B), gwm293[21-23,25,27] (5AS), gwm304a[21-23,25] (5AS) and wmc397[15,25,27] (6BS),

gwm608[15,27] (6B). The populations in which the markers were used are indicated by symbols in Fig. 1 and Fig. 2: Infinity/ND3085 (-), Infinity/ND744 (x) Alsen/Helios (+).

The PROC MIXED procedure of SAS[30] was applied to disease and toxin data with lines and replicates random. The *t*-test was applied to disease and toxin least square means classified by marker molecular variant to determine which loci contributed resistance.

RESULTS AND DISCUSSION

Fusarium head blight was present in all environments. For example, the Infinity/ND3085 overall test-mean disease index ranged from a low of 24.6 ± 13.0 at the U of M Carman to a high of 33.7 ± 17.0 at Ottawa. The line with the highest disease index ranged from 47.0 at Charlottetown to 76.5 at Ottawa. The other populations showed similar overall test-mean disease indexes, the highest being 54.1 ± 15.0 for the Infinity/ND744 test at AAFC Carman.

Markers were selected at loci previously reported to be associated with Sumai 3 FHB resistance[20-22,24-27,31]. Rarely were markers polymorphic on parents of all crosses, requiring different markers to be used to represent a given locus in a particular population. All selected markers were significant at least at the 0.05 level in at least one experimental site, for at least one disease measure. The Infinity/ND744 population was unrepresented with respect to the 6B locus and the Alsen/Helios population with respect to the 2D and 4D loci. Somers et al[31] summarized reported resistance from Sumai 3 on 3BS, 5A, 6B, 3AL, 6AS and 7D. Yang[27] found resistance to different disease attributes of Fusarium associated with chromosomes 2D, 3B, 4A, 4D, 5A, 6B and 7B as well as epistatic interactions with other loci involving other chromosomes. Among the three Sumai 3 derived sources of resistance, we found 3B, 5A and 2D to be major contributors of resistance (Fig. 1 and Fig. 2). The markers on 4D and 6B indicated weak contribution of these loci (Fig. 2). This could be because either alleles at these loci contribute little resistance or the markers are too distant to be of value.

The *gwm608* marker mapped closer to resistance according to Yang[27], but this marker was only weakly associated with severity and index measures of disease in the Infinity/ND744 population (Fig. 2). In contrast, the *gwm349*, which mapped further from resistance, affected incidence, severity and index in the Infinity/ND3085 population indicating ND3085 contributed the allele for resistance. Indications are that ND744 does not contribute a strong resistance allele at the 2D locus.

Based on consistent and/or strong responses with at least one marker, ND3085, ND744 and Alsen contribute resistance at the 3B and 5A loci. Some markers, such as

XSTS3B-66, were weakly associated with resistance in the same chromosomal region as other markers that were strongly associated (*gwm533*) with resistance (Fig. 1). This is likely related to the distance of the marker from the gene. Some markers were weakly associated with resistance in one population but showed a strong association in another, such as with *gwm285* which showed a weak association in the Infinity/ND3085 population, but a strong association in the Infinity/ND744 population (Fig. 1). This may be related to population size and the difference in number of cross-overs sampled between populations. If the marker is marginal, a population with few cross-overs may maintain a significant association whereas another population by chance may have enough cross-overs to appear to disrupt the association. For the same reason
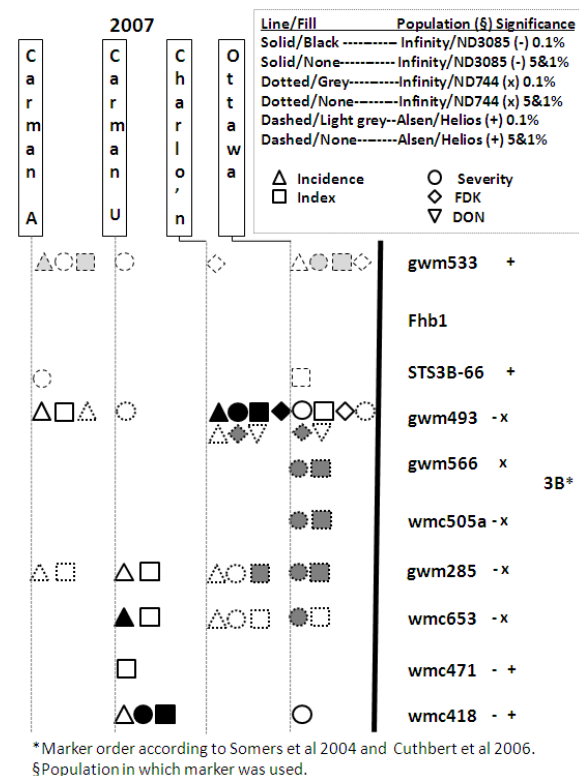
Fig. 1. Chromosome 3B significant *t*-test results for markers at the 1 to 5% level are represented by unfilled shapes and at the 0.1% level by filled shapes for FHB incidence, severity, index, *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) for populations Infinity/ND3085, Infinity/ND744 and Alsen/Helios for locations AAFC Carman MB, U of M Carman MB, Charlottetown PEI and Ottawa ON in 2007.

one marker, such as *wmc418*, could show a strong association with resistance at the 3B locus in disease testing at one location, but another marker, for example *wmc505a*, at the locus shows a strong association when the test is grown in another location. Apparently alleles for resistance will function in one environment and not another. For example, the ND744 allele marked by *gwm566* only produced measureable resistance in Ottawa and not Charlottetown nor Carman.

The *wmc418* marker was highly significant in the U of M Carman environment in the Infinity/ND3085 population, while *gwm493* showed little association with resistance in this environment. Yet *gwm493* was highly significant for several measures of disease at Charlottetown. McCartney et al[25] refer to two loci on chromosome 3B, one on the short arm and one near the centromere. The *gwm493* is a short arm marker and *wmc418* is near the centromere. A similar result was produced with the Infinity/ND744 population. Indications are that ND3085 and ND744 have active resistance alleles at both loci but Alsen does not. The 3B short arm locus contributed to reduction in FDK, DON, incidence and severity, whereas the centromere locus contributed to reduction in only incidence and severity. In general environment influenced the disease response relative to all markers reported here.
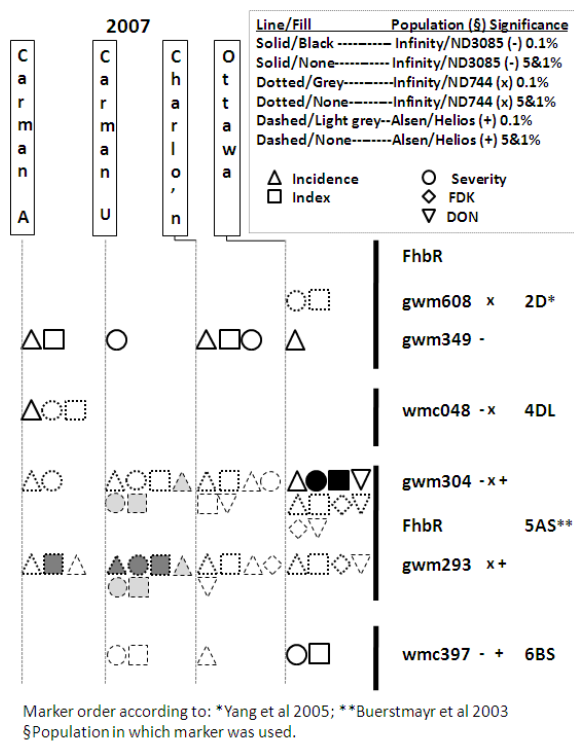

Marker order according to: *Yang et al 2005; **Buerstmayr et al 2003
§Population in which marker was used.

Fig. 2. Chromosome 2D, 4DL, 5AS and 6BS significant *t*-test results. See Fig. 1 for details.

Other than the short arm of 3B, the only other locus to influence FDK and DON was on chromosome 5A. Influence on DON generally only occurred when there was also a significant effect on FDK. There was only one example of FHB index being significant without either incidence or severity also being significant. Index contributes little additional information when incidence and severity are already considered.

In conclusion, ND3085 contributed resistance at loci on chromosome 2D, 5AS, 3B near the centromere and on the short arm, while ND744 contributed resistance at the two 3B loci and the 5AS locus. Alsen contributed resistance at the short arm 3B and 5AS loci.

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