Association mapping of UG99 resistance in a diverse durum wheat population

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INTRODUCTION

Association mapping (AM) is a complementary strategy to bi-parental mapping for describing associations between genotype and phenotype in crop plants (Somers et al. 2007). An attractive feature of AM is the ability to perform marker trait associations in well phenotyped breeding populations and locally adapted varieties. Association mapping has proven to be an effective strategy to identify marker-trait associations for important traits in wheat (Breseghello and Sorrells, 2006), including resistance to diseases (Crossa et al. 2007; Tommasini et al. 2007).

Stem or black rust, caused by *Puccinia graminis* f. sp. *tritici* Eriks. & Hennis has caused severe losses to hexaploid wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* var *durum*) production. Stem rust has been controlled effectively through the use of genetic resistance. However, the identification of stem rust race TTKS (UG99) is of great concern because this race has broad virulence to most *Sr* genes (Jin et al. 2007) and threatens global wheat production. Studies of the virulence of TTKS on hexaploid wheat varieties are increasing but data on the virulence of TTKS on durum wheat and the availability of molecular markers for selection for resistance is lacking. The objectives of this study were to survey virulence of TTKS on a collection of global durum wheat varieties and to perform association mapping to identify genomic regions that could be the target of marker-assisted selection and further genetic dissection in relevant genetic mapping populations.

MATERIALS AND METHODS

AM Population and Molecular Analysis– Ninety-six diverse durum wheat cultivars and breeding lines collected from breeding programs in Canada, Argentina, Australia, France, Italy, Germany, Mexico, Morocco, United States, New Zealand, Russia, Iran and Spain formed the AM population. Genotyping was performed on an ABI 3100 capillary electrophoresis using M13-labeled microsatellites. A total of 241 microsatellite (SSR) markers were used to amplify 245 loci (Somers et al. 2007).

Trait Analysis and AM Mapping –Resistance to stem rust race TTKS was assessed in a field trial at Njoro, Kenya in 2007. UG99 seedling tests using race TTKS were also performed in replicated greenhouse trials. Prior to AM analysis, population structure was assessed using the program STRUCTURE v.2 (Pritchard et al, 2000). Structure parameter settings were: linkage model, allele frequencies correlated, burn-in length 10,000, and 10,000 repetitions. The highest likelihood of the data was observed for K=5 and a Q matrix was estimated as the average of five runs for K=5. Using field severity and incidence ratings and seedling infection types, marker-trait associations were determined using a general linear model in TASSEL version 2.0.1 (Yu et al. 2006) with the Q-matrix as covariates. For structure and marker-trait associations, rare alleles (frequency <5%) were either combined into a single genotypic class if their combined frequency was greater than 5%, or scored as missing data. Significance of associations between loci and disease data was based on an F-test, at a significance level of $P \leq 0.01$, corrected for by performing 10,000 permutations.

RESULTS AND DISCUSSION

In the field trial in Kenya, infection responses ranged from susceptible to resistant with a high proportion of lines rated as resistant (26%) or moderately resistant (18%) (Table 1). The majority of durum varieties derived from Spanish breeding programs were rated moderately susceptible to stem rust. Varieties and breeding lines from Canadian and USA breeding programs were moderately resistant or resistant (Table 1).

In greenhouse seedling tests, 23% of lines were highly susceptible to TTKS, with the majority being from Italy (Table 1). There was a poor relationship between field and seedling reactions, with many lines rated susceptible at the seedling stage displaying good field resistance to stem rust. Likewise, many breeding lines and varieties susceptible in field screening displayed good resistance to TTKS in greenhouse testing (Table 1). This suggests the presence of independent seedling and adult plant resistance genes in this population. Alternatively, it is known that other stem rust races were present in the Njoro field trial, making it difficult to correlate field and greenhouse reactions.

Association mapping was performed on both field and seedling data, and several chromosome regions were identified that were significantly associated with either field severity and infection response, or seedling infection type (Table 2).

Table 1. Seedling infection type (IT) and field severity (Sev) and infection responses (IR) on adult plants in a field nursery at Njoro, Kenya.

Variety/Line	Origin	Sev	IR	IT	
BONAERENSE COMENAY	Argentina	1	R	13-	S
BONAERENSE QUILACO	Argentina	10	I	33+	S
BONAERENSE VALVERDE	Argentina	5	I	3+ -	S
BUCK AMBAR	Argentina	5	R	;1-	R
BUCK TOPACIO	Argentina	10	I	;1	R
920334	Australia	20	MSS	;1-	R
940030	Australia	60	S	;1=	R
940435	Australia	10	I	;1-	R
940955	Australia	20	I	;1-	R
950090	Australia	30	MSS	3+ -	S
950329	Australia	30	I	33+	S
950844	Australia	20	I	;1-	R
TAMAROI	Australia	5	R	;1-	R
WOLLAROI	Australia	1	R	;1-	R
9661-AF1D	Canada	15	I	1+ -	R
9661-CA5E	Canada	20	I	12-	R
AC AVONLEA	Canada	30	I	1+ -	R
AC MELITA	Canada	5	R	0;	R
AC MORSE	Canada	5	RMR	12-	R
AC NAVIGATOR	Canada	5	R	;1-	R
AC PATHFINDER	Canada	10	I	12-	R
CDC Verona	Canada	20	MSS	;1-	R
COMMANDER	Canada	1	R	0;	R
D24-1773	Canada	10	R	;1-	R
DT 513	Canada	20	MSS	;1-	R
DT 532	Canada	5	R	;1+	R
DT 536	Canada	20	I	;1=	R
DT 691	Canada	10	MS	;1	R
DT 695	Canada	5	R	0;	R
DT 696	Canada	5	R	1- 1	R
DT 704	Canada	20	RMR	;1-	R
DT 705	Canada	15	RMR	1+ -	R
DT 707	Canada	10	RMR	1+ -	R
DT 709	Canada	5	RMR	;1-	R
DT 710	Canada	5	R	1	R
DT 711	Canada	1	R	0;	R
KYLE	Canada	1	R	1+ -	R
NAPOLEON	Canada	1	R	0;	R
STRONGFIELD	Canada	20	MS	;1	R
CARIOCA	France	10	I	;1-	R
RABD 93-40	France	5	MR	;1-	R
TETRADUR	France	1	R	0;	R
DURABON	Germany	20	MS	3+ -	S
DURAFIT	Germany	20	I	;1-	R
44616	Iran	5	RMR	;1-	R
44721	Iran	5	I	;1-	R
CRDW 17	Iran	10	MSS	2+ -	R
D-73-15	Iran	5	R	;1	R
BRONTE	Italy	30	MS	33+), 1/6	S
CICCIO	Italy	10	I	2+ -	R
COLOSSEO	Italy	60	S	3+ -	S
DEMETRA	Italy	15	I	12+	R
DUILIO	Italy	5	R	3+ -	S
FLAVIO	Italy	5	RMR	23+	S
FORTORE	Italy	5	RMR	23	S
GIANNA	Italy	20	RMR	;1-	R
GRAZIA	Italy	10	RMR	;1-	R
IRIDE	Italy	20	I	;1-	R
LESINA	Italy	60	S	13	S
MONGIBELLO	Italy	5	R	12	R
NEDDA	Italy	10	S	12-	R
PARSIFAL	Italy	10	MS	23+	S
SIMETO	Italy	5	RMR	5/6 (;2+)	R
SVEVO	Italy	30	MSS	;1=	R
TRESOR	Italy	10	RMR	;1-	R
VARANO	Italy	10	I	3+ -	S
GREEN 27	Mexico	70	S	33+	S
GREEN 34	Mexico	20	I	3+	S
NACORI C 97	Mexico	30	MSS	1+ -	R
DHTON 1	Morrocco	30	I	23+	S
GIDARA 17A	Morrocco	30	I	33+	S
MARJAK	Morrocco	5	R	;1-	R
ARRIVATO	New Zealand	5	MSS	;1-	R
CFR5001	New Zealand	20	I	1- 2	R
CHAKINSKAYA 226	Russia	40	MS	33+	S
AGRIDUR	Spain	5	R	;1	R
ALTAR-AOS	Spain	10	I	;1	R
ARCOBALENO	Spain	30	MSS	;1-	R
ARIESOL	Spain	30	MSS	;1-	R
BORLI	Spain	20	MSS	;1-	R
CAMACHO	Spain	30	MSS	12+	R
GALLARETA	Spain	20	MSS	;1-	R
MEXA	Spain	20	MS	;1-	R
VITRON	Spain	20	I	4	S
D940027	USA	5	RMR	;1=	R
D940098	USA	5	RMR	;1	R
D95580	USA	30	S	3- 3	S
DUREX	USA	5	RMR	;1-	R
KOFA	USA	10	R	;1=	R
KRONOS	USA	1	R	;1-	R
LANGDON	USA	30	MSS	1+ -	R
LANGDON[DIC6B]	USA	5	RMR	0;	R
OCOTILLO	USA	5	R	;1-	R
PIERCE	USA	1	R	0;	R
PLAZA	USA	5	R	;1-	R
WESTBRED 881	USA	10	MR	;1-	R

Four genomic regions significant for both field and seedling data were identified on 1B, 2A, 6A, and 7A (Table 2), and these chromosomes house known *Sr* genes. Two regions were identified on chromosome 7A, one distal to the centromere, and a second at *gwm276* (Table 2). *Sr22* is linked to *gwm276*, and that gene is effective against TTKS (Jin et al., 2007). *Sr13* resides on 6A and is effective against TTKS and is derived from durum wheat variety "Khapli". Marker *gwm617* was significantly associated with TTKS resistance (Table 2) and is likely marking *Sr13* as they are both located on the distal region of 6AL. *Sr14* is also derived from "Khapli" has been localized distal to the centromere on 1B, and provides intermediate resistance TTKS (Jin et al. 2007). The c*fd48* (Table 2) locus on 1B associated with field and seedling resistance is also located distal to the centromere and is likely detecting variation at *Sr14*.

Table 2. Chromosome regions and significance of markers associated with field stem rust severity (Sev), and infection response (IR) and seedling infection type (IT) determined in greenhouse trials.

Chrom.	**Position (cM)**	**Marker**	**Sev**	**IR**	**IT**
1A	6	*gdm133*	0.016	0.020	ns
1B	40	*cfd48*	0.019	0.011	0.005
2A	16	*wmc382*	ns	ns	0.017
	55	*gwm95*	ns	ns	0.031
	60	*gwm372*	0.031	0.018	0.043
	85	*cfd168*	ns	ns	0.014
3B	39	*wmc808*	ns	ns	0.002
	150	*gwm340*	0.014	0.016	ns
4B	38	*barc20*	0.012	0.010	ns
5A	138	*gwm216*	0.041	ns	0.000
	153	*wmc727*	0.002	0.006	ns
5B	84	*wmc537*	0.003	0.002	ns
5B	82	*Cdu1*	0.007	0.008	ns
6A	95	*gwm617*	0.031	0.019	0.008
7A	40	*wmc283*	0.018	0.011	0.035
7A	83	*gmw276*	0.016	0.012	0.043
7B	143	*cfa2040*	ns	ns	0.000

Barc20 on 4B (Table 2) was significantly associated with variation in disease resistance and is linked to the lipoxygenase gene *Lpx-B1.1*. Lipoxygenase is known to play a role in disease resistance and enzyme activity has been reported to increase in wheat treated with a rust fungal elicitor (Bohland et al. 1997). Gene *SrTmp* from winter wheat cultivar 'Triumph 64' is effective against TTKS (Jin et al., 2007) and that gene is also believed to reside on 4B. More research is required to determine if barc20 is linked to *SrTmp*-derived resistance.

Several markers on 2A were significant and *Sr* genes, *Sr21, Sr32, Sr34 and Sr38*, are located on that

chromosome. *Sr32* is effective against TTKS at both the seedling and adult plant stages (Jin et al. 2007). Only *gwm372* was significant for both seedling and adult plant resistance and maybe associated with *Sr32*, but further testing would be required to validate this hypothesis.

In the Kenya field nursery, "Langdon" was rated as 30MSS (Table 1). In contrast, its near isogenic line "Langdon[DIC6B]", which contains a substituted 6B chromosome for *T. dicoccoides*, was rated 5RMR. These results suggest that an effective gene(s) for *Sr* resistance exists on 6B from *T. dicoccoides.* However, no 6B markers were associated with resistance in the AM population (Table 1).

Association mapping proved effective at identifying genomic regions associated with TTKS resistance in durum wheat, but these regions should be validated to determine their effectiveness in marker assisted selection programs. We are in the process of developing mapping populations from selected lines used in this study that appear to have unique *Sr* genes based on the haplotypes identified at significant loci (Table 2). In addition, the poor relationship between seedling and adult plant resistance needs to be further examined to identify those regions which will provide broad-spectrum resistance, particularly at the development stage when plants are most likely to become infected.

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REFERENCES

Bohland C, Balkenhohl T, Loers G, Feussner I, and Grambow H. (1997) Differential Induction of lipoxygenase isoforms in wheat upon treatment with Rust Fungus Elicitor, Chitin Oligosaccharides, Chitosan, and Methyl Jasmonate. Plant Physiol. 114, 679-685.

Breseghello F. and Sorrells, M.E. (2006) Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. Genetics 172:1165–1177.

Crossa J, Burgueno J, Dreisigacker S, Varga M, Herrera-Foessel SA, Lillemo M, Singh RP, Trethowan R, Warburton M, Franco J, Reynolds M, Crouch J, and Ortiz R. (2007) Association analysis of historical wheat germplasm using additive genetic covariance of relatives and population structure. Genetics 177: 1889-1913.

Jin Y, Singh RP, Ward RW, Wanyera R, Kinyua M, Njau, P, Fetch T, Pretorius Z A, and Yahyaoui, A. (2007) Characterization of seedling infection types and adult plant infectionresponses of monogenic Sr gene lines to race TTKS of *Puccinia graminis* f. sp. *tritici.* Plant Dis 91:1096-1099.

Pritchard, J.K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Somers D, Isaac P, and Edwards K. (.2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109, 1105-1114.

Somers DJ, Banks T, DePauw RM, Fox S, Clarke JM, Pozniak C, and McCartney C (2007) Genome-wide linkage disequilibrium analysis in bread wheat and durum wheat. Genome 50:557 – 567.

Yu, J., Pressoir, G., Briggs, W.H., Bi, I.V., Yamasaki, M., Doebley, J.F., McMullen, M.D., Gaut, B.S., Nielsen, D.M., Holland, J.B., Kresovich, S., and Buckler, E.S. 2006. A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nature Genetics 38:203-208.