

OTL Validation for Agronomic Traits on 3A Chromosome of Winter Wheat (Triticum aestivum) Using **Recombinant Inbred Chromosome Lines**



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INTRODUCTION

RESULTS AND DISCUSSION

Quantitative trait loci (QTLs) that control agronomic traits such as grain yield and its components are the most important economic traits for wheat (Triticum aestivum L.) improvement. Based on a previous study conducted using reciprocal chromosome substitution lines between winter wheat cultivars 'Wichita' (WI) and 'Cheyenne' (CNN), recombinant inbreed chromosome lines (RICLs) developed for the 3A chromosome in the CNN background CNN(RICLs-3A) indicated the presence of a major grain yield QTL that explained up to 28% of the phenotypic variance (Berke et al., 1992a, b; Campbell et al., 2003).

In the current study a population of WI(RICLs-3A) where the RICLs involve WI and CNN chromosome 3A in WI background was used to validate the OTLs detected in the CNN(RICLs-3A) population. Effectively, WI(RICLs-3A) is a mirror population to the previously studied CNN(RICLs-3A).

Therefore, the objectives of this study were to (1) identify and map QTLs for eight agronomic traits: (2) evaluate OTL x environment interaction (OEI) by comparing the consistency of OTLs detected in individual environments and: (3) determine the presence of common QTLs between the current population, WI(RICLs-3A), and the previous mapping population, CNN(RICLs-3A).

MATERIALS AND METHODS

A population of 90 WI(RICLs-3A) doubled haploid (DH) lines of the cross between cultivar Wichita (WI) and chromosome substitution line WI(CNN3A) were used to investigate OTLs for grain yield and other agronomic traits. The WI(RICLs-3A) population, parents and checks were grown in six environments (year x location) during the 2007/2008-2008/2009 cropping season at four sites in Nebraska (Lincoln, Mead, North Platte and Sidney). The experiment was evaluated in an incomplete block design with three-replications where each replication consisted of 20 incomplete blocks of 5 plots.

The agronomic traits evaluated include days to flowering (DF), plant height (PH), grain vield (GY), grain volume weight (GVW), 1000-kernel weight (TKW), kernels per spike (KPS), kernels per square meter (KPSM), and spikes per square meter (SPSM). Phenotypic traits were analyzed following the PROC MIXED procedure of the SAS version 9.1 (SAS Institute, 2004). Prior to the combined analysis, homogeneity of error variances was checked with the Bartlett's Chi-square test as outlined by Gomez and Gomez (1984).

MAPMAKER/ EXP3.0 program (Lander et al. 1987) was used for marker diagnostics and to determine the linkage groups. The map for the OTLs analysis was constructed from a single linkage group of 26 SSR markers. Identification of OTLs was performed using WinQTL cartographer v.2.5 (Statistical Genetics, NCSU, Raleigh, NC).

Table 1. compon	Correlatio ent traits or	n coeffi ı WI(RI	cients fo CLs-3A)	or agrono) mappir	omic, yi 1g popul	eld and y lation	vield	0.0 1.2 2.3 4.0 1.6 barc321 barc57 barc12 wmc532 wmc532 wm218
	PH	GY	GVW	TKW	KPS	KPMS	SPM2	35.9 barc86 38.0 \ [wmc640 - Parion 1
DF	0.62063**	-0.12**	-0.49**	-0.26**	-0.69**	-0.02ns	0.45**	39.4 39.9 barc356 GYLD, FD, PH, GV
PH		-0.03 ^{ns}	-0.51**	-0.23**	-0.52**	0.08**	0.42**	40.5 barc67 41.6 wmc3881
GY			0.49**	0.44**	0.22**	0.87**	0.47**	46.6 stm6362 51.5 hbg284 Region 2
GVW				0.47**	0.49**	0.29**	-0.13**	55.1 wmc428 GYLD, FD, PH, GVW, T 56.8 barc1060
TKW					0.45**	-0.06*	-0.33**	60.4 hbg227 61.0 gwm497
KPS						0.03%	-0.68**	65.9 cta2193 66.9 wmc559
KPMS							0.69**	71.0 / nog491 78.1 gwm155
"Sign	ificant at 0.05 level o	of probability	** Significa	int at the 0.01 l	ievel of probat	ility ns=not	significant	93.0 cfa2076 95.5 wmc3882 100.2 bart021

The ANOVA of all eight agronomic traits for the individual environments and for the combined analysis were highly significantly different (P≤0.01) (data not shown). Significant correlations were observed between grain yield and most of the yield components. Grain volume weight and yield component traits such as 100-kernel weight, kernels per spike, kernels per square meter, and spikes per square meter were all positively correlated with grain yield (Table 1). The parents showed significant differences for most of the phenotypic traits (Table 2).

OTLs were detected for seven of the eight agronomic traits (Table 3, Fig. 1). These QTLs explained from 12 % (for spikes per square meter) to 53 % (for grain volume weight) of the phenotypic variance of each trait. A grain yield QTL detected in the combined analysis explained 19 % of the phenotypic variance and the substitution of CNN allele for a WI allele decreased grain yield by 86 kg ha-1 (Table 3). This grain yield QTL is believed to be the major grain yield QTL detected in CNN(RICLs-3A) population as it mapped to a similar position (Fig. 1 and Table 3). In addition, a major QTL that affected days to flowering with 23 % of R² value was identified at North Platte during the 2007/2008 cropping season (Table 3). This flowering date QTL overlapped with plant height QTL (31 % R2 value on across environment) that was detected in more than two environments (Fig. 2). Also there was an observable phenotypic difference in flowering days of the two parents and cultivar Chevenne (Fig. 3).

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				Falenis	Standard		-	RICLS3A		
Trait name	Environment	wi	WI(CNN3A)	Estimate	Error	Pr > t	Mean	Range	deviation	
	LNK_08	152.9	154.8	-2.0	0.48	<.0001	152.9	150.0 - 155.0	1.31	
David to florencia a	NP_08	153.5	155.5	-2.1	0.46	<.0001	153.8	152.0 - 156.8	0.98	
Days to nowening	LNK_09	142.8	144.5	-1.7	0.45	0.0003	145.0	140.2 - 145.0	1.10	
	MD_09	144.8	147.5	-2.7	0.47	<.0001	146.7	141.9 - 146.7	1.28	
	NP 09	145.6	148.3	-2.7	0.40	<.0001	147.7	145.2 - 149.9	0.94	
	Combined	148.1	150.15	-2.1	0.35	<.0001	148.5	139.0 - 158.0	4.46	
	LNK 08	106.6	116.8	-10.2	1.89	<.0001	104.4	97.0 - 116.0	4.62	
	NP 08	118.3	123.5	-5.3	2 42	0.0317	117.5	104.0 - 123.3	3.63	
Plant height (cm)	LNK 09	83.9	98.0	-14.1	2.22	<.0001	90.7	82.6 - 105.0	4.90	
	MD 09	96.8	101.4	-4.6	2 40	0.056	97.9	892-1100	4.74	
	NP 09	113.0	123.7	-10.8	2.17	<.0001	118.7	109.5 - 127.6	3.96	
	SD 09	94.1	100.5	-6.4	2.18	0.0039	91.3	751-1044	4.84	
	Combined	102.1	110.6	-8.4	1.93	< 0001	104.1	737-1295	11.80	
	INK 08	2796.7	2187.6	609.1	346.44	0.0809	2395.1	1574.0 - 3629.0	490.38	
	NP 08	4799.8	4378.1	421.8	251.74	0.0961	4670.5	2344 5 - 4903 2	410 71	
Grain yield (kg ha-1)	1 NK 09	4147.5	3500.3	647.2	263.75	0.0154	3967.7	3332.8 - 4503.2	317.86	
	MD 09	4073 7	3510.9	562.8	288.02	0.0528	3584.8	2476.6 - 4388.5	387.64	
	NP 09	3518.9	3062.1	456.8	211 37	0.0324	3860.9	2664.2 - 3031.1	260.03	
	SD 09	3812.0	3285.1	527.8	170.03	0.0039	4053.8	2004.2 0001.1	200.00	
	Combined	2002.0	3210.2	542.0	171.00	0.0016	2612.0	1201.0 6020.0	770.60	
	L NIC OR	3003.2	3313.2	1.0	0.70	0.0010	72.7	71.0 75.0	110.00	
Grain volume weight	ND 08	74.0	74.0	1.5	0.75	0.0203	78.2	F0.0 70.5	1.00	
(kg hL-1)	INP_08	/0.8	74.9	1.9	0.98	0.0045	76.3	70.2 92.6	1.20	
	LIVE_05	02.1	30.0	1.5	0.01	0.0040	75.0	79.2 - 02.0	0.07	
	MD_09	74.8	73.5	1.4	0.86	0.0963	75.2	70.1 - 76.1	1.00	
	NP_09	74.2	73.0	0.4	0.00	0.5354	74.7	71.8 - 75.7	0.76	
	SD_09	75.6	74.1	1.5	0.50	0.004	70.7	74.3 - 77.0	0.74	
	Combined	/6.3	75.0	1.4	0.45	0.0025	76.0	67.1-65.6	2.90	
100 kornel weight	LINK_08	32.8	33.9	-1.1	2.10	0.0045	30.5	26.9 - 37.0	1.90	
(gram)	NP_08	38.5	38.5	0.0	1.11	0.9915	39.0	34.4 - 41.1	1.41	
	LNK_09	38.0	39.6	-1./	1.3/	0.2281	38.3	34.3 - 41.5	1.31	
	MD_09	39.4	37.1	2.2	1.59	0.1595	38.2	32.3 - 41.4	1./1	
	NP_09	31.6	31.8	-0.2	1.92	0.9165	34.7	28.7 - 35.0	1.45	
	SD_09	34.2	35.7	-1.4	1.22	0.2403	36.5	31.8 - 37.3	1.25	
	Combined	35.7	36.1	-0.4	0.74	0.6057	35.8	17.5 - 45.4	3.62	
	LNK_08	26.2	22.2	4.0	2.12	0.0643	23.7	17.7 - 28.1	2.09	
Kernels ner snike	NP_08	21.7	23.4	-1.7	1.59	0.2785	23.1	19.7 - 25.9	1.34	
	LNK_09	32.7	34.9	-2.2	2.10	0.2898	36.1	28.0 - 39.4	2.31	
	MD_09	32.2	31.1	1.2	1.91	0.5446	31.2	28.5 - 36.7	1.77	
	NP_09	25.4	22.7	2.7	1.65	0.1063	23.3	19.6 - 27.2	1.55	
	SD_09	19.5	21.9	-2.3	1.80	0.196	24.3	20.6 - 26.8	1.48	
	Combined	26.3	26.0	0.3	0.94	0.7461	26.4	15.2 - 45	5.50	
	LNK_08	8504.6	6697.8	1806.7	1212.32	0.1384	6618.2	5142.0 - 12097.0	1574.01	
Kernels per square	NP_08	12472.0	11317.0	1155.0	763.93	0.1328	12028.0	6864.1 - 13019	954.80	
	LNK_09	10965.0	8853.5	2111.5	739.39	0.005	10397.0	8189.0 - 12417.0	861.31	
	MD_09	10465.0	9472.1	992.9	830.21	0.2337	9344.1	6847.9 - 11379.0	909.6	
	NP_09	11131.0	9663.6	1467.4	924.45	0.1147	11024.0	8150.1 - 12573.0	875.0	
	SD_09	11148.0	9199.2	1948.8	629.99	0.0024	11083.0	8489.2 - 11817.0	650.3	
	Combined	10797.0	9196.4	1600.6	504.50	0.0016	10112.0	3884.0 - 21728.0	2017.0	
	LNK_08	330.9	302.1	28.8	69.04	0.6772	281.1	194.5 - 564.7	80.82	
Spikes per square	NP_08	578.2	493.7	84.5	52.02	0.1064	522.3	323.6 - 616.0	51.5	
meter	LNK_09	337.4	253.7	83.6	30.90	0.0076	290.0	244.6 - 422.9	33.8	
	MD_09	326.7	314.2	12.5	28.46	0.6611	302.5	191.2 - 376.6	33.3	
	NP_09	431.9	418.1	13.8	49.06	0.3716	479.2	375.6 - 555.0	46.4	
	SD_09	575.9	426.8	149.2	47.01	0.0019	456.4	337.9 - 529.9	38.3	
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Table 3	Summary of OTLs detected in 90	WI(RICLs-3A) population for agronomic	vield and vield components traits at
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aach anu	ironments and combined analysis		

						Most significant	% variation	Additive	Peak position	Contributor
No.	QTL	Trait name	Environment	LOD score	Flanking markers	marker	explained (R*)	effect1	(cM)	parent
1	QFIt.neb-3A.1	Days to flower (days)	NP08	5.1	barc86 - stm6352	wmc640	23	-0.45	38.0	CNN
2	QFIt.neb-3A.2	Days to flower (days)	NP08	3.8	stm6352 - hbg284	hbg284	19	-0.39	51.1	CNN
3	QHLneb-3A.1	Plant height (cm)	NP08	5.2	gwm218 - wmc664	wmc640	23	-1.61	38.0	CNN
4	QHt.neb-3A.2	Plant height (cm)	NP08	3.9	stm6352 - hbg284	hbg284	19	-1.45	51.1	CNN
5	QHtneb-3A.3	Plant height (cm)	LNK09	3.9	stm6352 - wmc559	barc1060	18	-1.96	56.8	CNN
6	QHLneb-3A.4	Plant height (cm)	MD09	3.1	wmc388.1 - gwm497	wmc428	15	-1.76	55.6	CNN
7	QHt.neb-3A.5	Plant height (cm)	NP09	5.5	gwm218 - wmc664	wmc640	25	-1.87	39.0	CNN
8	QHLneb-3A.6	Plant height (cm)	SD09	5.3	hbg284 - gwm497	barc1060	26	-2.20	57.8	CNN
9	QHtneb-3A.7	Plant height (cm)	Combined	7.1	barc86 - barc67	wmc640	31	-1.62	38.0	CNN
10	QHt.neb-3A.8	Plant height (cm)	Combined	6.3	stm6352 - hbg284	stm6352	28	-1.55	50.1	CNN
11	Qyld.neb-3A.1	Grain yield (kg ha ⁻¹)	LNK08	2.8	barc86 - wmc388.1	wmc640	13	173.00	38.0	WI
12	Qyld.neb-3A.2	Grain yield (kg ha ⁻¹)	NP09	3.4	stm6352 - cfa2193	barc1060	17	101.00	58.8	wi
13	Qyld.neb-3A.3	Grain yield (kg ha-1)	Combined	4.0	barc86 - stm6352	wmc640	19	86.60	38.0	WI
14	Qyld.neb-3A.4	Grain yield (kg ha ⁻¹)	Combined	3.6	stm6352 - hbg284	hbg284	17	82.40	51.1	WI
15	QGvw.neb-3A.1	Grain volume weight (kg hL-1)	LNK08	8.9	barc86 - stm6352	wmc388.1	39	0.52	42.6	WI
16	QGvw.neb-3A.2	Grain volume weight (kg hL ⁻¹)	NP08	3.8	wmc664 - stm6352	wmc388.1	18	0.45	41.6	WI
17	QGvw.neb-3A.3	Grain volume weight (kg hL ⁻¹)	LNK09	13.5	barc86 - stm6352	wmc388.1	49	0.44	41.6	WI
18	QGvw.neb-3A.4	Grain volume weight (kg hL-1)	MD09	8.4	gwm218 - wmc640	barc86	35	0.58	35.6	WI
19	QGvw.neb-3A.5	Grain volume weight (kg hL-1)	NP09	8.2	barc86 - stm6352	wmc640	35	0.42	36.9	WI
20	QGvw.neb-3A.6	Grain volume weight (kg hL-1)	NP09	7.6	stm6352 - wmc428	hbg284	32	0.40	51.1	WI
21	QGvw.neb-3A.7	Grain volume weight (kg hL-1)	Combined	14.9	barc86 - stm6352	wmc388.1	53	0.39	41.6	WI
22	QGvw.neb-3A.8	Grain volume weight (kg hL ⁻¹)	Combined	14.0	stm6352 - wmc428	hbg284	50	0.39	51.5	WI
23	QTgw.neb-3A.1	Thousand-kernel weight (gm)	LNK08	4.1	barc67 - stm6352	wmc388.1	19	0.80	41.6	WI
24	QTgw.neb-3A.2	Thousand-kernel weight (gm)	NP08	3.4	stm6352 - wmc559	gwm497	16	0.53	61.0	WI
25	QTgw.neb-3A.3	Thousand-kernel weight (gm)	Combined	4.9	barc86 - wmc664	wmc640	21	0.43	38.0	WI
26	QTgw.neb-3A.4	Thousand-kernel weight (gm)	Combined	4.3	stm6352 - hbg284	hbg284	19	0.41	51.1	WI
27	QKps.neb-3A	Kernel per spike (number)	SD09	2.8	wmc640 - barc356	wmc664	13	-0.52	39.4	CNN
28	QSsm.neb-3A.1	Spike per square meter (number)	NP09	3.4	gwm218 - wmc640	barc86	16	18.03	35.9	WI
29	QSsm.neb-3A.2	Spike per square meter (number)	Combined	2.7	barc86 - barc356	wmc640	12	9.20	38.0	WI





Figure 2. Days to flowering and plant height QTLs after composite interval mapping overlapping at the same region (2 years at North Platte and across environments)

Figure 3 Days to flowering at North Platte between cultivar Chevenne, Wichita and WI(CNN3A) during 2007/2008 cropping season

CONCLUSIONS

The results of this study confirm the previously mapped major QTLs in the CNN(RICLs-3A) population on the 3A chromosome were the same major QTLs detected in the current WI(RICLs-3A) population and have affected grain yield and its related traits. The WI 3A alleles in the previous CNN(RICLs-3A) study showed an increase in grain yield (66 kg ha-1) and, as expected, the CNN 3A alleles in this WI(RICLs-3A) study had caused a reduction of grain yield (86 kg ha-1). Given the consistent expression of most of these major QTLs in different backgrounds and environments, the marker information could be useful in future breeders' selection programs. Populations derived from reciprocal chromosome substitution lines, such as the one used in this study, are very useful tool for validating QTLs on specific chromosome and also have additional advantage in limiting one-way chromosome substitution in determining interactions between chromosomes.

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