

Mapping QTLs for quality characters in durum wheat (*Triticum turgidum* L. ssp. *durum*)

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ABSTRACT

In durum wheat, thousand-kernel weight (TKW), test weight (TW), grain protein content (GPC), gluten strength and yellow pigment content (YPC) are important traits for end use quality. A recombinant inbred line population developed from a cross PDW 233 / Bhalegaon 4 was analysed for these parameters across major durum growing environments in India and the molecular linkage map was developed to locate the QTLs responsible for variation in these traits. In composite interval mapping (CIM), five different QTLs associated with gluten strength, as measured by SDS-sedimentation volume were identified. *Q_{Sv.macs-1B.1}* flanked by marker interval *Xgwm550 - Glu-B3* was identified at $LOD \geq 4.19$ in five environments and explained 9.18% to 40.66% of phenotypic variance of the trait. Along with glutenin coding loci *Glu-B1* and *Glu-B2* on 1B, loci on chromosomes 3B and 4B were also found to be associated with gluten strength. For YPC, five QTLs were identified on chromosomes 1A, 3B, 5B, 7A and 7B across five environments. The strongest one, *Q_{Yp.macs-7A}*, located on the distal part of 7AL, explained 55.22% of the variation in the trait. Total seven QTLs, each accounting for 5.86% to 9.85% of variation in GPC, were identified, out of these, two each were detected on chromosomes 1B and 7A and one each on 2A, 5A and 7B. Three QTLs for TKW, each explaining 9.25% to 18.89% of variation, were located on chromosomes 2A, 4B and 6B, whereas, four QTLs influencing the TW with phenotypic variance ranging 7.78% - 11.58%, were detected on chromosomes 1A, 2A and 7A. QTLs identified for these important quality traits will be useful in marker assisted breeding for improvement of durum wheat.

INTRODUCTION

Durum wheat ranks eighth in the world among all cereals with more than 21 million hectares area under cultivation and nearly 65 million tons production. It is mainly used for the preparation of semolina and pasta, local breads, couscous and chapati. It is a well-established fact that the best pasta is obtained from durum wheat semolina. Therefore, importance is given to developing the durum cultivars with better milling and pasta making quality and increased yield. The quality of durum wheat is a multidisciplinary concept and changes according to the end user. The main commercial and technological characteristics considered by millers and pasta makers are kernel properties like hectoliter weight, thousand kernel weight, vitreousness

as well as milling yield, carotenoid content, quality and quantity of gluten and grain protein content. Consumers are concerned about firmness of cooked pasta, cooking loss, stickiness and colour of pasta. The main objective of this study was to investigate the genetic basis of these components, and provide molecular markers to implement in improving the quality of durum wheat products.

MATERIAL AND METHODS

A population of 140 $F_{2:7}$ RILs was developed from the cross of durum wheat PDW 233 (YAV'S'/TEN'S') and Bhalegaon 4 (Landrace). The RILs were sown in Randomized Block Design with two replications each at three locations namely, Pune in Peninsular Zone [2001-02 (EI); 2002-03 (EIII); 2003-04 (EV); 2004-05 (EVI); 2005-06 (EVII) and 2006-07 (EVIII)], Indore in Central Zone [2002-03 (EII)] and Karnal in North Zone [2002-03 (EIV)] to study the effect of environment on all the traits under study. Each year \times location combination will be further called "environment". The GPC was measured by near-infrared spectroscopy with a Technicon 400 Infra-analyzer (Technicon Corp, NY), whereas, gluten strength was determined using modified SDS-microsedimentation test (Dick and Quick, 1983). Yellow pigment content was estimated by the modified AACC method (Santra et al., 2003). Marker analysis, gluten protein separation and QTL identification was carried out as described in Patil *et al.*, 2008.

RESULTS AND DISCUSSION

Map construction

Total 173 SSR, 8 ISSR, 4 TRAP markers (*Xari*), 2 locus specific markers for gluten proteins, 2 SCARs, 2 STS and 2 morphological markers (Awn colour-*Bla* and glume pubescence-*Hg*) were polymorphic between the parents and mapped in the population. The map comprises 201 markers spanning a distance of 2296.8 cM forming 13 linkage groups representing all the chromosomes except 3A. Maximum 29 markers were mapped on chromosome 1B, whereas, minimum 8 markers on chromosomes 2B and 6A. Average marker density was 11.43 cM/marker and average map length was 176.67 cM/chromosome.

Gluten protein variation in parents

In gluten protein analysis, parents showed the same null allele at *Glu-A1* locus but differed in alleles at *Glu-B1*, *Glu-B2* and *Glu-B3* loci (Fig. 1a). PDW 233 had 7+8 band and Bhalegaon 4 had band 20 coded by *Glu-B1*.

Analysis of LMW-GS showed presence of a band coded by *Glu-B2* in PDW 233 (*Glu-B2a*) and its allelic variant null (*Glu-B2b*) in Bhalegaon 4. Among the two subunits coded by *Glu-B3*, the faster migrating subunit was different between PDW 233 and Bhalegaon 4. The SDS-PAGE analysis of gliadins revealed six polymorphic bands between parents, of which three were mapped on chromosome 1A and one each on chromosome 1B and 6B (Fig. 1b). In Acid-PAGE analysis, the gliadins coded by *Gli-A1*, *Gli-A2* and *Gli-B2* showed differences between parents along with another gliadin fraction mapped on 1A (Fig. 1c).

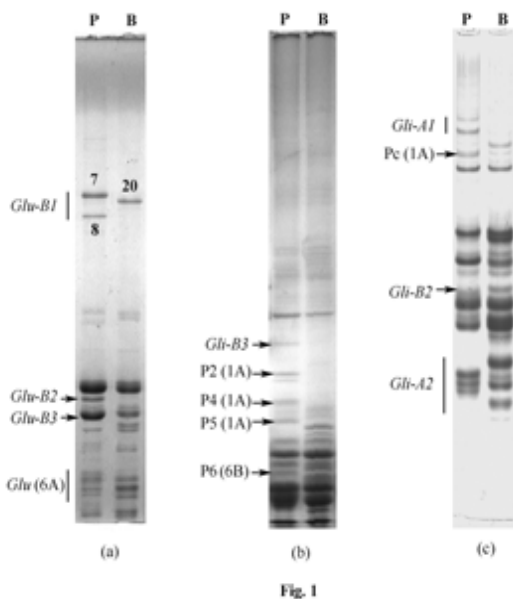


Fig. 1 Glutenin and gliadin profiles of PDW 233 (P) and Bhalegaon 4 (B).

QTL detection

SDS-Sedimentation volume (SV): In composite interval analysis, five different QTLs associated with SV were identified (Table 1). Three of them were located on chromosome 1B in close vicinity to glutenin coding loci *Glu-B1*, *Glu-B2* and *Glu-B3*. *QSV.macs-1B.1* flanked by marker interval *Xgwm550 - Glu-B3* was identified at $LOD \geq 4.19$ in all the five environments and explained 9.18% to 40.66% of phenotypic variance of the trait. Another QTL, *QSV.macs-1B.2*, flanked by *Glu-B2 - XGlu-B3* was detected in all the environments except EII and explained 6.76% to 10.01% of the variation in the trait. The third QTL, *QSV.macs-1B.3*, located on long arm of chromosome 1B near to glutenin coding locus *Glu-B1* and accounting for up to 19.63% of variation in SV, was detected in all the environments except EV. Increased SV was observed due to PWD 233 allele at all the three QTLs on chromosome 1B. Two more putative QTLs, *QSV.macs-3B* and *QSV.macs-4B* were associated with the trait only in one environment. Their negative

additive effect, suggested the contribution of Bhalegaon 4 towards SV.

Yellow pigment content (YPC): Five different QTLs linked to yellow pigment content were identified. The major one, *QYp.macs-7A*, located between loci *Xubc807₉₉₀* and *XE33M62* on 7AL, was consistent over the five environments, explained between 22.6 and 55.2% of the variation in the trait and the favourable allele was contributed by PDW 233. The markers were subsequently converted to SCAR markers (scar807; scar3362) for the ease of MAS (Patil *et al.*, 2008). Two more QTLs, *QYp.macs-7B*, flanked by *Xari4* and *Xgwm46* and *QYp.macs-1A*, linked to *Xubc835₆₁₀-1A* near the centromere were detected only in two environments EI & EV and EIII & EV, respectively. Two minor QTLs for yellow pigment content, *QYp.macs-5B* and *QYp.macs-3B* were detected only in one environment. Only *QYp.macs-1A* showed negative additive effect indicating the contribution of Bhalegaon 4, the inferior parent, for the trait.

Grain protein content (GPC): Total seven QTLs associated with GPC were identified in three environments EII, EIII and EV, whereas, no QTL was detected for GPC in EIV. *QGpc.macs-7B* at marker interval *Xgwm46 - Xbarc231.4* was the only QTL detected in more than one environment (EII and EIII) which explained up to 9.64% of variation in GPC. Chromosome 1B and 7A were found to harbour two QTLs each for GPC, while, one QTL each was observed on chromosome 2A, 5A and 7B. The QTLs on chromosome 7A showed positive additive effect indicating the rise in GPC due to PDW 233 allele at these QTLs. Rest of the QTLs were contributed by Bhalegaon 4.

Test weight (TW): The trait was influenced by four QTLs, two of them were located on chromosome 1A and one each on chromosome 2A and 7A. *QTw.macs-2A* was consistent for two environment and explained upto 11.58% of variation in TW.

Thousand kernel weight (TKW): Three QTLs responsible for variation in TKW were identified, out of them *QTKw.macs-2A*, flanked by *Xgwm71.2 - Xubc835.4* was consistently observed in both the environments. Two more QTLs on chromosome 4B and 6B explained upto 18.89% of variation in the trait.

Importance in breeding: Developing durum wheat with superior end-use quality is a tough task because of wide range of end products. The quality traits are also difficult to manipulate by conventional breeding due to pronounced effect of environment on these traits. Therefore, extensive research is warranted to understand the genetic basis of end-use quality traits of durum wheat. The SCAR markers (scar807; scar3362) and glutenin coding loci *Glu-B1*, *Glu-B2* and *Glu-B3* will be helpful for selection of breeding line with increased YPC and better gluten strength, respectively. A QTL flanked by *Xgwm71.2 - Xubc835.4* on chromosome 2A has pleiotropic effect on TW and TKW. QTLs identified in present study will be useful to improve milling and pasta making quality of Indian durum wheat.

Table 1. QTLs for quality traits detected in RILs from PDW 233 / Bhalegaon 4

Trait	Environment	QTL	Marker Interval	LOD score	R ² %	Additive effect
SDS-sedimentation volume (SV)	EI	<i>QSv.macs-1B.1</i>	<i>Xgwm550 – Glu-B3</i>	7.40	17.15	1.85
		<i>QSv.macs-1B.2</i>	<i>Glu-B2 – XGlu-B3</i>	4.16	7.39	1.22
		<i>QSv.macs-1B.3</i>	<i>Glu-B1 – Xksum219</i>	2.76	5.76	1.01
	EII	<i>QSv.macs-1B.1</i>	<i>Xgwm550 – Glu-B3</i>	16.41	40.66	6.21
		<i>QSv.macs-1B.3</i>	<i>Glu-B1 – Xksum219</i>	9.62	19.63	4.32
		<i>QSv.macs-3B</i>	<i>Xgwm566 – Xbarc218</i>	2.27	4.92	- 2.13
	EIII	<i>QSv.macs-1B.1</i>	<i>Xgwm550 – Glu-B3</i>	7.23	15.47	3.06
		<i>QSv.macs-1B.2</i>	<i>Glu-B2 – XGlu-B3</i>	5.74	10.01	2.56
		<i>QSv.macs-1B.3</i>	<i>Glu-B1 – Xksum219</i>	4.78	8.61	2.24
	EIV	<i>QSv.macs-1B.1</i>	<i>Xgwm550 – Glu-B3</i>	6.09	18.62	3.17
		<i>QSv.macs-1B.2</i>	<i>Glu-B2 – XGlu-B3</i>	3.26	6.76	1.91
		<i>QSv.macs-1B.3</i>	<i>Xksum219 – Xksum76</i>	4.26	12.04	2.42
		<i>QSv.macs-4B</i>	<i>Xgwm6 – Xgwm155</i>	2.77	11.58	- 2.32
	EV	<i>QSv.macs-1B.1</i>	<i>Xgwm550 – Glu-B3</i>	4.19	9.18	1.89
		<i>QSv.macs-1B.2</i>	<i>Glu-B2 – XGlu-B3</i>	2.97	6.94	1.63
Grain protein content (GPC)	EII	<i>QGpc.macs-1B.1</i>	<i>Xcfa2158.2 – Xwmc406</i>	2.36	6.56	- 1.71
		<i>QGpc.macs-7A.1</i>	<i>Xcfa2028 – Xcfa2174</i>	2.27	9.85	2.16
		<i>QGpc.macs-7B</i>	<i>Xgwm46 – Xbarc231.4</i>	2.22	5.86	- 1.63
	EIII	<i>QGpc.macs-2A</i>	<i>Xgpcw2333 – Xcfa2099</i>	2.58	6.58	- 1.72
		<i>QGpc.macs-1B.2</i>	<i>Xgwm582.2 – Xcfa2129</i>	2.31	6.26	- 2.08
		<i>QGpc.macs-7A.2</i>	<i>Xbarc231.1 – Xbarc154</i>	2.11	7.76	2.31
		<i>QGpc.macs-7B</i>	<i>Xgwm46 – Xbarc231.4</i>	3.87	9.64	- 2.59
EV	<i>QGpc.macs-5A</i>	<i>Xgwm129 – Xbarc100</i>	2.20	7.56	- 2.59	
Yellow pigment content (YPC)	EI	<i>QYp.macs-7A</i>	<i>Xscar3362 – Xscar807</i>	7.85	22.61	0.67
		<i>QYp.macs-7B</i>	<i>Xari4 – Xgwm46</i>	3.06	8.75	0.41
		<i>QYp.macs-5B</i>	<i>Xgwm408 – Xbarc232</i>	2.16	7.31	0.37
	EII	<i>QYp.macs-3B</i>	<i>Xksum76.2 – Xbarc218</i>	2.34	6.87	0.36
		<i>QYp.macs-7A</i>	<i>Xscar3362 – Xscar807</i>	20.70	55.22	1.33
		<i>QYp.macs-7A</i>	<i>Xscar3362 – Xscar807</i>	13.78	43.39	1.11
	EIII	<i>QYp.macs-1A</i>	<i>Xubc835₆₁₀ – XDuPw38</i>	3.71	5.00	- 0.41
		<i>QYp.macs-7A</i>	<i>Xscar3362 – Xscar807</i>	7.26	25.79	0.95
	EIV	<i>QYp.macs-7A</i>	<i>Xscar3362 – Xscar807</i>	10.75	25.87	0.98
		<i>QYp.macs-7B</i>	<i>Xari4 – Xgwm46</i>	2.43	8.01	0.51
		<i>QYp.macs-1A</i>	<i>Xubc835₆₁₀ – XDuPw38</i>	3.08	6.12	- 0.44
	Test Weight (TW)	EVIII	<i>QTw.macs-2A</i>	<i>Xgwm71.2 – Xubc835.4</i>	3.66	11.58
<i>QTw.macs-1A.1</i>			<i>Pc – P4</i>	3.27	11.27	0.81
EVII		<i>QTw.macs-1A.2</i>	<i>Xubc835.1 – XDuPw38</i>	2.79	7.78	0.66
		<i>QTw.macs-7A</i>	<i>Xcfa2174.1 – Xgwm573</i>	2.49	7.85	0.66
		<i>QTw.macs-2A</i>	<i>Xgwm71.2 – Xubc835.4</i>	3.30	8.98	0.92
Thousand Kernel Weight (TKW)	EVI	<i>QTKw.macs-6B</i>	<i>Xgwm361 – Xbarc79</i>	2.23	9.25	1.71
		<i>QTKw.macs-4B</i>	<i>XDuPw23 – Xgwm165.2</i>	4.02	18.89	- 2.45
	EVIII	<i>QTKw.macs-2A</i>	<i>Xgwm71.2 – Xubc835.4</i>	3.56	12.25	1.97
		<i>QTKw.macs-2A</i>	<i>Xgwm71.2 – Xubc835.4</i>	2.80	9.55	2.38

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