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Identification of molecular markers associated with yield and quality traits for Argentinean durum wheat breeding programs

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Abstract. Developing cultivars with high grain yield and optimal quality traits for pasta production is the main goal of durum wheat breeding programs worldwide. This work summarize the main results of our research group using a RIL mapping population (UC1113 x Kofa) evaluated in six environments from Argentina to detect QTLs and linked markers for yield and yield components, phenology, gluten strength, protein and color. QTLs affecting yield and yield components were mapped on chromosomes 3BS (*gwm493*), 2BS (*cfa2201*), 3AL (*ksm28-wmc428*) and 4AL. The first two QTL also affect heading date and/or flowering time. QTLs affecting flour yellow color (Fb*) were located on 4AL (*wmc219*), 6AL (*wmc553*) and 7BL (*cfa2040-barc1073*). The QTLs on 6AL and 7BL plus a 7BS QTL (*barc72*) also affect yellow pigment content (Ypc). Another important QTL for Ypc and Fb* was linked to *Psy-B1* gene. The deletion of the *Lpx-B1.1* from Kofa resulted in a significant decrease of lipoxigenase activity and in an improving in pasta color. For gluten strength, the most important and stable QTL was located on 1BL (Glu-B1) and two additional regions were located on 6AL (*wmc553*) y 6BL (*gwm219*). Two QTLs located on 3BS (*barc147-gwm493*) and 7BL (*cfa2040-barc1073*) were found as affecting protein content. The flanking markers of the QTLs detected in this work could be efficient tools to select superior genotypes to improve the Argentinean durum wheats.

Keywords. QTL – Yield – Quality – MAS – Durum wheat.

Identification de marqueurs moléculaires associés aux caractères liés au rendement et à la qualité dans les programmes d'amélioration du blé dur argentin

Résumé. *Le développement de cultivars à haut rendement en grain et avec des caractères de qualité optimaux pour la production de pâtes est l'objectif prioritaire des programmes de sélection du blé dur dans le monde entier. Ce travail résume les principaux résultats obtenus par notre groupe de recherche à l'aide d'une population de cartographie RIL (UC1113 x Kofa), évaluée dans six différents environnements en Argentine, pour détecter des QTLs et des marqueurs liés au rendement et à ses composantes, la phénologie, la force du gluten, les protéines et la couleur. Les QTLs affectant le rendement et ses composantes ont été cartographiés sur les chromosomes 3BS (gwm493), 2BS (cfa2201), 3AL (ksm28-wmc428) et 4AL. Les deux premiers QTL affectent également la date d'épiaison et/ou la date de floraison. Les QTLs affectant la couleur jaune de la farine (Fb*) sont situés sur 4AL (wmc219), 6AL (wmc553) et 7BL (cfa2040-barc1073). Les QTL sur 6AL et 7BL plus un QTL 7BS (barc72) affectent également la teneur en pigment jaune (Ypc). Un autre QTL important pour Ypc et Fb* est lié au gène Psy-B1. La délétion de la Lpx-B1.1 de Kofa a entraîné une diminution significative de l'activité lipoxygénase et une amélioration de la couleur des pâtes. Pour la force du gluten, le QTL le plus important et stable est situé sur 1BL (Glu-B1) et deux autres régions sont situées sur 6AL (wmc553) et 6BL (gwm219). On a observé que deux QTL situés sur 3BS (barc147-gwm493) et 7BL (cfa2040-barc1073) affectent la teneur en protéines. Les marqueurs flanquant les QTL détectés dans cette étude pourraient être utilisés efficacement pour sélectionner des génotypes supérieurs afin d'améliorer les blés durs argentins.*

Mots-clés. *QTL – Rendement – Qualité – MAS – Blé dur.*

I – Introduction

Durum wheat (*Triticum turgidum* L. ssp. *durum*) is mainly used to produce pasta because its grains are the only ones, among the cereals, able to produce semolina. The aptitude of semolina for pasta production is conferred by the particular characteristics of its endosperm storage proteins

that comprise the gluten matrix. A strong gluten and high protein content are conductive to the production of dough with excellent rheological properties for pasta making. Another important quality trait for durum wheat is the yellow color in milling products, due mainly to high carotenoid pigment content and low lipoxygenase activity. Developing cultivars with high grain yield and optimal quality traits for pasta production has been the main goal of durum wheat breeding programs worldwide. Since 2004 our group works in mapping quantitative trait loci (QTLs) associated with these traits in order to implement MAS in Argentinean breeding programmes (Carrera *et al*., 2007; Picca *et al*., 2008; Garbus *et al*., 2009; Conti *et al*., 2011; Roncallo *et al*., 2012). The starting point of our work in this field was done using a RIL population resulting from a cross of foreign germplasm. Genetic dissection using QTL analysis on local environments allowed to find genomic regions expressed in our durum wheat area. The present work resumed the main results of our research group using this bi-parental mapping population (UC1113 x Kofa) in order to detect QTL and linked markers for grain yield and yield components, phenology, gluten strength, protein and color. Field evaluations were performed in different locations of the main durum wheat area of the Province of Buenos Aires, Argentina.

II – Material and methods

1. Plant material

The mapping population consisted of 93 F9 recombinant inbred lines (RILs) obtained by single seed descent from the cross between the line UC1113 and the variety Kofa (Carrera *et al*., 2007).

2. Field trials

The 93 RILs and the parental lines were sown during two consecutive years (2006 and 2007) in three locations from Argentina (Cabildo [CA], Barrow [BW] and Balcarce [BC]). Field trials were sown following a randomized complete block design with three replications using experimental plots of 3 m^2 in size and 150/ m^2. Each year x location combination was considered an environment in the statistics analysis. Agronomical management of fertilization with nitrogen and phosphorus was performed in two applications, at presowing or sowing and tillering, according to local practices and doses for each experimental field. All traits were determined in the three replications of each genotype in each environment.

3. Quality traits

Whole-wheat flour was obtained by milling grains with an UDY experimental cyclonic mill (UDY Corporation) with a 1 mm sieve.

Gluten: Sedimentation volume test (Sv) was determined according to the technique described by Dick and Quick (1983) using 1 g of whole wheat flour.

Protein: Grain protein content (Gpc) was evaluated and expressed in percentage using Near-Infrared Transmission (NIRT) (Infratec 1226, Tecator, Suecia) according to IRAM procedure 15.879 and based upon 12% moisture content.

Color: Lipoxygenase activity (LOX) and yellow pigment content (Ypc) were determined. LOX extraction and substrate preparation were performed as described by Carrera *et al*. (2007) based on McDonald (1979) and Surrey (1964). Yellow pigments were analyzed using the protocol of Fares *et al*. (1991) as described in Roncallo *et al*. (2012). Flour yellow color (Fb) was measured with a MINOLTA chromameter (CIE L*a*b*).

4. Industrial Quality

Test weight (Tw) was obtained using a Schopper balance.

5. Yield and yield components

Grain yield (Yld) from each entire plot was obtained by weighing the harvested clean grains using a harvest machine (Kg/ha). Thousand grain weight (Tgw) was recorded by weighing two samples of 100 grains from each plot. Yield components were obtained from ten plants randomly collected from the central row of each plot after harvest maturity, expressed as mean value. The value per plant was calculated as an average of all ears by plant. Harvest index (Hi) was obtained as the ratio between the total weight of grains per plant and the weight of the plant. Spikelet number/ear (Sne) was obtained as the average number of total spikelets/ear, counting the number of spikelets in all the ears/plant. Grain weight/ear (Gwe) was determined by weighing the grains from each ear of the plant. Grain number/ear (Gne) was calculated as the product of the weight of grains/spike (Gwe) and the average weight of one grain which was obtained from the thousand grain weight. Spike fertility (Sf) was calculated as the ratio number of fertile spikelets/ear (Fse) and number of total spikelets/ear (Sne). Grain number per fertile spikelets (Gnfs) was calculated by dividing the grain number per ear with fertile spikelets per ear from each individual plant. Grain number per total spikelets (Gnts) obtained as the product of grain yield per ear and the individual grain weight obtained from thousand grain weight.

6. Morphological and phenological traits

Plant height (Ph) was measured as the distance from the edge of separation of the stem from the root to the tip of the spike (cm). Peduncle length (Pd) was measured as the distance from the last internode to the base of the spike (cm.). Heading date (Hd) was determined as the number of days between emergence and heading (Zadoks stage 55). Flowering time (Flt) was calculated as the number of days between emergence and flowering (Zadoks stage 65) (Zadoks *et al*., 1974).

7. QTL mapping

Genetic map: A total of 269 markers, including 23 SNP markers, were arranged on 14 linkage groups covering a total length of 2,140 cM, in this population (Zhang *et al*., 2008).

QTLs for lipoxygenases were mapped using a map constructed with 83 RILs based on the markers of Zhang *et al*. (2008) enriched with 44 AFLP, 9 RAPD, two isozymes and one storage protein. The genetic map was constructed using the software GQMOL (Schuster and Cruz, 2004) as was described in Picca *et al*. (2008).

Mapping method: QTL mapping was performed by the CIM method using the Windows QTL-Cartographer software v.2.5 (Wang *et al*., 2004) as was described in Roncallo *et al*. (2012).

III – Results

The main QTLs mapped using the UC1113 x Kofa mapping population are summarized in Table 1. QTL analysis showed several pleiotropic regions affecting correlated traits. Most of the positive alleles for quality were provided by Kofa whereas the positive alleles for yield came from UC1113.

The main QTLs affecting yield and yield components were located on chromosome arm 3BS, closest linked to the SSR marker *gwm493*. Kofa had the positive allele for this QTL that explained a maximum R^2 of 38% for yield in BC 2006 and also affected several yield components (Table 1).

Table 1. Main QTLs affecting yield, yield components and quality traits mapped in a durum wheat RILs population in six environments from Argentina.

QTL -Chr. arm	Closest marker	Positive allele	LOD score	Additive effect	R^2 (%)	Peak position (cM)	Environment	Individual envir. No.	Pleiotropic effect (positive allele)
QYld.cerz-1BL1	*BE443797_436*	UC1113	5.1	-122.65	13.4	47.1	Mean	1	Fb (K), Ypc (K), Gwe (U), Fse(U)
QSv.cerz-1BL2	*Glu-b1*	Kofa	17.6	5.19	46.2	81.8	Mean	6	
QYld.cerz-2BS	*cfa2201*	UC1113	7.1	-150.45	23.0	18.8	CA 2006	2	Hd(K),FltK),Sne(K), Gpc(U)
QYld.cerz-3AL	*Ksm28-wmc:428*	UC1113	6.9	-136.65	18.0	66.4	Mean	3	Gne (U), Hi(U)
QYld.cerz-3BS	*gwm493*	Kofa	8.1	149.43	20.7	13.0	Mean	2	Tw(K),Gne(K),Gwe(K), Ph(K),
QYld.cerz-4AL1	*dupw4-barc170*	Kofa	4.9	119.09	14.3	44.2	Ca 2006	1	PdL(K), Hd(KU), Flt(U), Gpc(U) Fb(U), Gpc(U), Sv(U), Hi(K), Gnfs(K)
QPh.cerz-4AL2	*wmc258-wmc718*	UC1113	5.9	-1.96	13.3	58.8	Mean	4	PdL(U), Gwe(U), Bpp(U)
QFb.cerz- 4AL3	*wmc219*	Kofa	4.0	0.24	10.6	126.2	Mean	3	
QLpx.cerz-4BS	*Lpx-B1.1*	Kofa	18.98	-	68.4	13.2	UC Davis	2 (2003-2004)	
QTgw.cerz-5BL	*BE495277_339*	UC1113	4.1	-0.69	13.2	73.3	Mean	2	Tw(U), Ypc(U), Gpc(U)
QYpc.cerz-6AL	*wmc553*	Kofa	10.5	0.43	29.9	65.4	Mean	5	Fb(K), Sv(K), Sne(K), Hd(K)
QTgw.cerz-6BL1	*BE604119_469 or wmc105*	Kofa	4.2	0.73	15.9	64.9	Mean	2	
QSv.cerz-6BL2	*gwm219*	Kofa	4.7	2.35	9.6	117.5	Mean	4	
QSv.cerz-7AS	*barc70*	Kofa	7.3	3.01	15.2	16.0	Mean	2	Hi(U), Sf(U)
QYpc.cerz-7BS	*barc72*	Kofa	4.1	0.27	9.5	59.2	BW 2007	2	Fb(K)
QGpc.cerz-7BL	*barc1073*	Kofa	4.3	0.21	15.6	185.6	Mean	3	Ypc(K), Fb(K), Sne(K)
QYpc.cerz-7BL2	*Psy-B1*	Kofa	2.9	0.24	7.9	195.5	Mean	1	Fb(K)

Yld= Yield, Sv= Sedimentation volume, Ph= Plant height,Fb= Flour b value, Lpx= Lipoxygenase activity, Tgw= Thousand grain weight, Ypc= Yellow pigment content, Gpc=Grain protein content,Gwe= Grain weight per ear,Fse=Fertile sp kelets per ear, Hd=Heading date,Flt= Flowering time, Sne=Sp kelets number per ear, Gne=Grain number per ear, Hi= Harvest index, Tw= test weight, PdL= Peduncle length, Gnfs= Grain number per fertile spikelets, Bpp= Total biomass per plant, Sf= spike fertility. Alleles: K= Kofa, U= UC1113. CA= Cabildo (Buenos Aires), BW= Tres Arroyos (Buenos Aires).

IV – Discussion

Height reduction (−55%) caused by the putative *Rht5* gene located on chromosome 3BS was reported in bread wheat (Rebetzke *et al*. 2012), associated with delayed flowering, lesser number of grains by spike and yield. The UC1113 allele for the 3BS QTL (*gwm493-cfd79*) showed a similar effect in our analysis for these traits. The QTL mapped on 2BS was located near on *Ppd-B1* gene based on the consensus map of Sommers *et al*. (2004) and Mohler *et al*. (2004).

The markers presented here are in process to validation using an association mapping population consisting on 167 entries. The validated advantageous alleles will be used for MAS in public and private breeding programs in Argentina.

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