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RESEARCH ARTICLE

Validation of Simple Sequence Repeats Associated with Quality Traits in Durum Wheat

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Abstract

Over recent years, quality has become an important commercial issue for durum wheat breeders. Modern breeding methods are most efficient for producing and supplying the best quality raw materials to the pasta industry. Here we assessed the effectiveness of molecular marker-assisted selection of quality traits in durum wheat. To this end, DNA and quality trait markers were jointly used to analyze quality-related traits in a durum wheat collection. A total of 132 durum wheat (*Triticum turgidum* ssp. *durum*) Mediterranean landraces, international lines, and Moroccan cultivars were analyzed for seven important quality-related traits including thousand-kernel weight (TKW), test weight (TW), gluten strength, yellow pigment (YP), and grain protein content (GPC). Additionally, 18 simple sequence repeat (SSR) markers previously reported to be associated with different quality traits were analyzed. Of these, 14 (78%) were polymorphic and four were monomorphic. There were between two and seven alleles per locus, with an average of four alleles per locus. The average phenotypic variation value (R^2) ranged from 2.81 to 20.43%. Association analysis identified nine markers significantly associated with TKW, TW, and YP, followed by eight markers associated with GPC, six markers associated with yellow index b, four markers associated with brightness L, and three markers associated with SDS-sedimentation volume. This study highlights the efficiency of SSR technology, which holds promise for a wide range of applications in marker-assisted wheat breeding programs.

Key words : marker validation, simple sequence repeat (SSR), quality traits, Triticum turgidum ssp. Durum

Introduction

Wheat breeders aim to increase grain yield (Duveiller et al. 2007) and improve grain quality to meet the requirements of an ever-increasing population. Quality refers to the desirability of the product and may include various physical and chemical parameters depending on the intended purpose. To predict processing and end-product quality, factors influencing wheat grain quality are broadly classified into two groups: physical and chemical. Physical characteristics include the 1000-kernel weight (TKW) and test weight (TW), while chemical characters include grain protein content (GPC), yellow pigment content (YP), and sedimentation test (Goutam et al. 2013). The major disadvantages of these traits reside in their methods of analysis, which are often time consuming and impose major constraints on breeders' resources (Howitt et al. 2007; Singh et al. 2012). Moreover, they are limited in number and are influenced by environmental factors (Winter and Kahl 1995). Therefore, reliable and efficient tools are urgently needed. The availability of various molecular markers associated with quality characteristics has aided

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the selection of desired traits with relative ease (Goutam et al. 2013). Due to their high polymorphism rate, co-dominant characteristics, selective neutrality, distribution across the genome, and cost and labor efficiency, microsatellites or simple sequence repeats (SSR[1]s) are suitable markers for detecting allele frequency within a population and for assessing population structure (De Vienne and Causse 1998; Gustafson et al. 2007; Maccaferri et al. 2003). To date, many SSR[1] markers designed to distinguish allelic variations within different quality parameters have been developed to screen for grain quality traits (Blanco et al. 2006; Patil et al. 2008b; Röder et al. 1998). However, due to the multigenic nature of many quality traits and the need to assess the effect of the environment on the trait, the discovery of molecular markers linked to phenotypic variation is only a preliminary step in establishing a marker-assisted selection program for genetic improvement (Suprayogi et al. 2009). Quantitative trait loci with large effects are very useful for trait improvement via marker-assisted selection. However, molecular markers linked to quantitative trait loci are being routinely developed using materials derived from bi-parental crosses such as F2, recombinant inbred lines (RILs), and double haploid (DH) populations on limited genetic backgrounds and covering only a few meiotic events (Caballero et al. 2008). In fact, the effectiveness of molecular markers needs to be validated by determining the target phenotype in independent populations and different genetic backgrounds; this is referred to as marker validation (Sharp et al. 2001). New approaches have recently been proposed to avoid spurious associations due to population structure in quantitative trait loci, such as investigating molecular marker association using germplasm collections of randomly sampled unrelated individuals (Flint-Garcia et al. 2005; Roy et al. 2006; Terracciano et al. 2013). Compared to quantitative trait locus analyses, association studies have the potential to directly mine the allelic diversity of genetic resources and identify alleles that are beneficial for the trait of interest (Haussmann et al. 2004; Maccaferri et al. 2006, 2010, 2011). Therefore, the present study aimed to validate 18 SSR[1] markers associated with quality traits using a collection composed of 132 durum wheat (*Triticum durum Desf.*) accessions including Mediterranean landraces, international lines from CYMMIT[2] and ICARDA[3], and Moroccan cultivars. This approach helps to resolve associations between genotype and phenotype that could aid durum wheat breeders to select important traits in earlier generations.

Materials and Methods

Plant materials

A total of 132 durum wheat accessions (*Triticum turgidum* L. var. *durum*) were analyzed. These included: 46 Mediterranean landraces (31 from Morocco) and 15 durum wheat landraces originating from six southern and eastern Mediterranean countries, 46 advanced lines obtained from CYMMIT[2], and 18 advanced lines obtained from ICARDA[3].

Table 1. ANOVA analysis of the quality traits for 132 durum wheat accessions from different origins

	Traits	TKW[a] (g)	TW[b] (KghL^{-1})	b[c]	L[d]	YP[e] (ppm)	GPC[f] (%)	SDS[g] (mL)
MEDL[h]	Mean	30.56^{b}	77.25^{c}	20.74^{a}	84.33^{a}	7.44^{a}	15.99^{a}	42.36^{c}
	SE	1.37	1.64	0.40	0.68	0.44	0.63	2.43
ML[i]	Mean	33.90^{ab}	79.80^{bc}	21.22^{a}	85.11^{a}	7.56^{a}	15.37^{a}	45.27^{bc}
	SE	0.79	0.99	0.23	0.47	0.15	0.41	1.24
IALC[j]	Mean	35.18^{a}	83.71^{a}	19.49^{b}	85.43^{a}	6.58^{b}	14.16^{b}	47^{ab}
	SE	1.21	0.63	0.24	0.55	0.21	0.28	0.87
IALI[k]	Mean	33.21^{ab}	82.59^{a}	19.16^{b}	78.06^{b}	6.49^{b}	14.14^{b}	50.12^{a}
	SE	0.94	1.02	0.53	2.75	0.26	0.26	1.59
MV[l]	Mean	34.85^{a}	81.97^{ab}	18.07^{c}	85.10^{a}	5.41^{c}	15.29^{ab}	43.04^{bc}
	SE	1.58	0.87	0.44	1.88	0.37	0.54	1.84
	LSD	3.75	2.76	1.00	3.51	0.80	1.18	4.32
	DF	4	4	4	4	4	4	4
	CV%	18.83	5.79	8.69	7.17	20.62	12.76	16.11
	MS	70.64*	153.28***	39.98***	195.93^{ns}	17.81***	12.85*	160.3*

Means by the same letter are not significantly different according to Tukey' Student used range test at $P \leq 0.05$; [a]1000-kernel weight; [b]test weight; [c] yellow index; [d]brightness, [e]yellow pigment content; [f]grain protein content; [g] SDS-volume sedimentation; [h]Mediterranean landraces; [i]Moroccan landraces; [j] CYMMIT lines; [k] ICARDA lines; lMoroccan variety.

Twenty-two Moroccan cultivars were also included. All accessions were kindly provided by the durum wheat breeding program of the National Institute of Agronomical Research of Rabat, Morocco. Chinese Spring wheat was used as control.

Experimental design

The quality trait assessment was conducted in trials at Allal Tazi (34°31'N, 6°19'W; INRA's research station) using an augmented block design. Each entry was sown in four rows 2.5-m long and spaced at 0.3 m, only the two rows in the middle were harvested. Standard agronomic management of soil preparation, fertilization, and weeding were applied, and the fertilizer used was 19-38-0 (N-P-K) complex applied at 150 kg ha^{-1} and amino nitrate (33.5% N) applied at 100 kg ha^{-1}.

Quality trait assessment

The quality parameters evaluated were: 1) gluten strength, determined by the SDS sedimentation test as described previously (American Association for Cereal Chemistry (AACC) 1984); 2) yellow pigment concentration, assayed using the AACC 14-50 modified method (Santra et al. 2003); 3) grain color, evaluated by measuring brightness (L[4]) and yellow index (b[5]) parameters with a Chroma Meter CR-400 reflectance colorimeter (Konica Minolta); 4) grain protein concentration (GPC), determined on grain from individual plots using a INFRANEO near-infrared reflectance spectrophotometer (NIRS); 5) test weight (TW), determined using an Aqua-TR (Tripette and Renaud Chopin); and 6) 1000-kernel weight (TKW).

DNA extraction and PCR[6] amplification

Leaves were collected from 2-3-week-old seedlings grown

Table 2. Pearson's correlation coefficients between quality traits in durum wheat accessions

	TKW[a]	TW[b]	b[c]	L[d]	YP[e]	GPC[f]	SDS[g]
TKW	1	1	1	1	1	1	1
TW	0.47***	-0.21*	-0.21*	-0.16*	0.12	0.01	
b	-0.24*	0.1	0.74***	0.1	0.06		
L	0.08	-0.35***	0.07	-0.09			
YP	-0.46***	-0.20*	-0.06				
GPC	-0.08	-0.01					
SDS	-0.07						

***Correlation is significant at the 0.0001 level; **Correlation is significant at the 0.001 level; * Correlation is significant at the 0.05 level. [a]1000-kernel weight; [b]test weight; [c]yellow index; [d]brightness, [e] yellow pigment content; [f] grain protein content; [g]SDS-volume sedimentation.

in a growth chamber, lyophilized, and 20 mg used for DNA extraction using the CTAB procedure as reported by Gale (2005). For molecular marker analysis, PCR[6] was performed in a final volume of 10 µL containing: 2 µL genomic DNA (50 ng), 2 µL 5×PCR[6] buffer (Promega, Madison, USA), 1 µL dNTPs (0.2 mM for each dNTP), 1 µL of each primer (10 pM), 0.2 µL $MgCl_2$ (1.5 mM), and 0.05 µL of Go-*Taq* DNA polymerase ($5U\mu L^{-1}$) (Promega); double distilled sterile water was added to a final volume of 10 µL. DNA amplification was carried out in a thermocycler (Eppendorf Mastercycler Gradient, Eppendorf, Hamburg, Germany). The thermocycling program was: initial denaturation at 94°C for 5 min followed by 30 cycles of 30 s at 94°C, 30 s at the annealing temperature (Table S2), 1 min at 72°C, and a final cycle of 5 min at 72°C. PCR[6] products were separated by 6% (w/v) denaturating polyacrylamide gel electrophoresis (Morgante and Olivieri 1993), and the gel was stained with silver nitrate as described by Benbouza et al. (2006).

Microsatellite loci analysis

Microsatellites were selected based on available information with respect to proximity to known quantitative trait loci that control durum wheat quality traits to directly validate the reliability of any associations (Table S3). Eighteen SSR[1] markers were chosen from the publicly available sets catalogued in the GrainGenes database (http://wheat.pw.usda.gov) and published by Patil et al. (2008b): CFA, CFD, and GPW (*Xcfa*, *Xcfd*, *Xgpw*) and Gatersleben wheat microsatellites (*gwm*) (Röder et al. 1998) were used: 10 *Xgwm* markers, developed at IPK Gatersleben (Institute of Plant Genetics, Germany) (Pestsova et al. 2000; Röder et al. 1998); three *Xwmc* markers from the Wheat Microsatellite Consortium (Gupta et al. 2002); one *Xcfa* marker developed at INRA Clermont-Ferrand, France (Guyomarc'h et al. 2002; Sourdille et al. 2003); and one UHW, one GPW, and one expressed sequence tag (EST)-SSR[1] *DupW*, developed by Eujayl et al. (2002) (Table S2). SSR[1] electrophoretic profiles were visualized directly. Allele types were coded "a" to "f" on each locus. The presence (1) or absence (0) of an allele was used to establish a binary matrix composed of 0s and 1s. From this matrix, 14/18 loci resulted in polymorphic alleles that could be scored as either 0 or 1. Chinese Spring wheat was used as a standard marker.

Table 3. Analysis of genetic differentiation between and within genotypes by AMOVA.

Source	df	SS	MS	Est. Var.	%	PhiPT[a]	P[b]
Between Pops[c]	4	182.297	45.574	1.416	16%		
Within Pops[d]	134	1029.940	7.686	7.686	84%		
Total	138	1212.237		9.102	100%	0.16	0.001

[a]PhiPT is based on standard permutation across the full data set; [b] Probability (rand data); [c]within populations; [d] between populations.

Statistical analysis

Analysis of molecular variance (AMOVA) was performed using GenAlexv.6.1 software (Peakall and Smouse 2006) to detect variations between and within foreign and national accessions and between landraces, advanced lines, and varieties. The quality traits data of the germplasm used were submitted to analysis of variance (ANOVA) and means were compared utilizing the Tukey test at 5% significance and Pearson Correlation coefficients (r) were determined between various characters. A Mixed Linear Model (MLM) was constructed in PowerMarker v3.25 software to investigate associations between quality-related traits and SSR[1] markers. The effect of each marker on total variance (R^2 values) was estimated using SAS v9.2.

Results

The analysis of variance exhibited significant differences ($P < 0.05$) among the germplasm analyzed, suggesting the existence of variability among the 132 genotypes for the seven quality traits (Table 1).

Comparison of the mean values of grain quality traits for the different typologies of durum wheat accessions (landraces, advanced breeding lines, and modern cultivars) showed a steady increase in TW, TKW, and gluten strength but a decrease in GPC, YP, and yellow index b (Table 1 and Fig. 1).

The Moroccan and Mediterranean landrace genotypes exhibited significantly higher yellow pigment concentrations (7.44 ± 0.44 ppm; 7.56 ± 0.15 ppm) compared to international lines 6.58 ± 0.21 and 6.49 ± 0.26 ppm) and Moroccan varieties (5.41 ± 0.37 ppm). Similar results were observed for yellow index b: the mean values were 20.74 ± 0.40 and 21.22 ± 0.23 within landraces followed by international lines (19.49 ± 0.24 and 19.16 ± 0.53) and Moroccan varieties (18.07 ± 0.44). No significant differences in Brightness L were observed between the different durum wheat typologies (Table 1).

Protein concentrations decreased from landraces to international lines, with the mean value dropping from 15.99 ± 0.63 and 15.37 ± 0.41%, respectively, in Moroccan and Mediterranean landraces to about 14.16 ± 0.28% and 14.14 ±

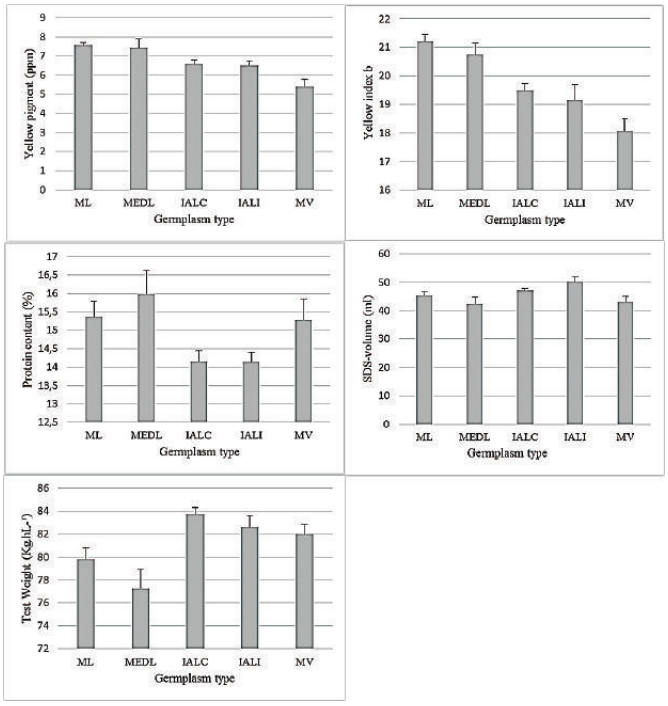

Fig. 1. Variability within the different typologies of durum wheat germplasm for the most crucial traits: Yellow pigments content, yellow index b, protein content, SDS-sedimentation volume and test weight. ML: Moroccan landraces, MEDL: Mediterranean landraces, IALC: CYMMIT lines, IALC: ICARDA lines, MV: Moroccan variety.

0.26% in CYMMIT and ICARDA lines, respectively; this trait remained high in Moroccan varieties (15.29 ± 0.54%). With respect to SDS sedimentation volume, the mean value was relatively higher in CYMMIT and ICARDA international lines (47 ± 0.87 mL and 50.12 ± 1.59 mL, respectively) compared to Moroccan and Mediterranean landraces (42.36 ± 2.43 mL; 45.27 ± 1.24 mL, respectively) and Moroccan varieties (43.04 ± 1.84 mL).

Regarding yield-related traits, both internationals lines (CYMMIT and ICARDA) and the Moroccan varieties exhibited higher TW values (83.71± 0.63 kg hL^{-1}; 82.59±1.02 kg hL^{-1} and 81.97 ± 0.87 kg hL^{-1}, respectively) compared to landrace genotypes (77.25 ± 1.64 kg hL^{-1}; 79.80 ± 0.99 kg hL^{-1}). Similarly, the mean values of the TKW were relatively higher in international lines and Moroccan varieties (35.18 ± 1.21 g; 33.21 ± 0.94 g and 34.85 ± 1.58 g, respectively) compared to landraces (30.56 ± 1.37 g; 33.90 ± 0.79 g).

Quality trait correlation analysis

Most Pearson's correlation coefficients between the seven characteristics were either positively or negatively significant (Table 2). Yellow pigment content was significantly positively correlated with yellow index b (0.74***) and negatively correlated with the test weight (-0.664**), TKW (-0.46***), and brightness L (-0.581**). Brightness L was negatively correlated with yellow index b (-0.21*). The yellow index b was negatively correlated with TKW (-0.24***) and TW (-0.21**). The test weight was positively correlated with TKW (0.47***) but negatively correlated (r = -0.20*) with the GPC.

Population diversity and marker polymorphisms

AMOVA analysis revealed that most of the total genetic variation (84%) occurred within durum wheat populations, while 16% was due to genetic differentiation between these populations. Therefore, the overall genetic differentiation between lines and cultivars was low (P = 0.001, PhiPT = 0.16) (Table 1). According to the electrophoretic patterns on denaturating polyacrylamide gels, a total of 56 alleles were scored across accessions. The number of alleles per locus ranged from two for *Xgwm471* and *Xgwm508* to seven for *Xcfa2174* (Table S4).

SSR[1] marker quality trait associations

The analysis showed that each of the seven traits was associated with one or more of the 14 polymorphic SSR[1] markers. The markers explained between 7.11% (SDS-sedimentation test) and 20.43% (test weight) of the total variation (R^2) for individual traits (Table 4).

A total of 14 SSR[1] markers were identified, each of which showed moderate-to-strong or strong-to-very strong associations with at least one of the seven traits detected by the mixed linear model (Table 4). For the remaining traits, the number of markers varied between two and nine. Nine markers were identified in association with TKW, TW, and YP, eight markers for GPC, six markers for b^5, four markers for L^4, and three markers for SDS-sedimentation volume. In contrast, of the 14 SSRs[1], *Xgwm344* was associated with TKW, TW, b^5, L^4, YP, and GPC. *Xcfa2174*, *Xgwm550,* and *Xgwm499* were associated with TKW, TW, YP, SDS-sedimentation volume and GPC. *Xgwm299* was associated with TW, TKW, YP, b^5, and GPC. *Xwmc522* and *Xgwm146* were both associated with TW, TKW, YP, and GPC. *Xgwm46* was associated with TKW, L^4, and GPC. However, *Xgwm508* was only associated with b^5, *XdupW38* was only associated with L^4, *Xgwm371* was associated with the two milling quality traits (TW and TKW), and *Xgwm471* was only associated with TW.

Quantitative trait loci analysis for quality traits in durum has been extensively studied (Blanco et al. 2011; Elouafi et al. 2001; Howitt et al. 2009; Mares and Campbell 2001; Patil et al. 2008b; Reimer et al. 2008). However, few studies have reported multiple markers. Therefore, we compared the markers identified in the present study with SSR[1] markers previously identified by linkage quantitative trait loci and association mapping analyses (Table S3). Of 14 markers, nine were associated or linked with the same traits (YP) identified in previous studies (Table S3). Conversely, other markers such as *Xgwm371*, which are supposed to be associated with SDS-sedimentation volume (Kerfal et al. 2010), were strongly and significantly associated with b^5. Similarly, *Xgwm508* was not associated with GPC (Olmos et al. 2003) but with YP. *Xgwm408* was associated with yellow color components (YP, b^5, and L^4), and *Xwmc283* were associated to two of the yellow color components (YP and b^5).

Table 4. List of SSR marker alleles significantly associated with quality traits (TKW, TW, YP, b, L, SDS, and GPC) detected in the collection by the mixed linear model and their allele effect .

Source	Marker	Allele	F-value[a]	R^{2b}	Allele effect[c]
TKW[d]	Xgwm371	a	11.01**	7.81	-
	Xgwm371	b	9.38**	6.73	+
	Xgwm550	a	7.83**	5.77	+
	Xgwm550	d	9.69**	7.04	-
	Xwmc522	a'	9.57**	6.91	-
	Xwmc522	b	4.23*	3.17	-
	Xwmc522	c	9.55**	6.89	+
	Xgwm299	a	9.07**	6.57	-
	Xgwm299	b	13.83**	9.69	+
	Xgwm46	a	4.53*	3.45	-
	Xgwm46	b	7.41**	5.51	+
	Xgwm499	a	8.53**	6.16	-
	Xgwm344	b	11.17**	7.91	+
	Xgwm344	c	4.51*	3.36	-
	Xgwm344	d	5.55*	4.10	-
	Xgwm146	b	5.37*	3.97	+
	Xcfa2174	f	7.01**	5.16	-
TW[e]	Xgwm471	a	4.56*	3.39	+
	Xgwm471	b	4.56*	3.39	-
	Xgwm371	a	3.83*	2.86	-
	Xgwm371	b	6.19*	4.54	+
	Xgwm550	a	10.01**	7.25	+
	Xgwm550	d	32.86***	20.43	-
	Xgwm550	c	7.96**	5.85	-
	Xwmc522	a'	31.66***	19.71	-
	Xwmc522	c	9.49**	6.85	+
	Xgwm299	a	3.84*	2.89	-
	Xgwm299	b	4.37*	3.28	+
	Xgwm499	a	13.11**	9.16	-
	Xgwm499	b	17.52***	11.88	+
	Xgwm344	a	6.97**	5.09	-
	Xgwm146	b	10.69**	7.60	+
	Xgwm146	d	7.88**	5.72	-
	Xcfa2171	a	5.76*	4.28	-
	Xcfa2171	b	8.59**	6.25	-
	Xcfa2171	c	8.97**	6.51	+
	Xcfa2171	f	11.14**	7.95	-
bf	Xgwm508	a	5.56*	4.08	-
	Xgwm508	b	5.56*	4.08	+
	Xwmc283	e	14.96**	10.32	-
	Xgwm299	a	6.07*	4.46	+
	Xgwm299	b	4.23*	3.15	-
	Xgwm344	b	7.26**	5.25	-
	Xgwm344	e	4.42*	3.26	+
	Xgwm408	a	3.78*	2.81	+
	Xgwm408	c	3.81*	2.83	-
	Xgwm146	b	4.37**	3.23	-
	Xgwm146	d	15.56**	10.62	+
Lg	Xgwm46	a	10.30**	7.45	-
	Xgwm344	d	7.52**	5.43	-
	Xgwm408	b	3.86*	2.87	+
	Xdupw38	b	5.05*	4.01	+
YP[h]	Xgwm550	d	8.43**	6.18	+
	Xwmc522	a'	3.86*	2.91	+
	Xwmc283	a	5.19*	3.87	-
	Xwmc283	e	9.99**	7.19	-
	Xgwm299	a	5.39*	4.01	+
	Xgwm299	b	4.98*	3.72	-
	Xgwm499	a	4.55*	3.38	+
	Xgwm344	a	4.49*	3.34	+
	Xgwm344	b	8.97**	6.45	-
	Xgwm408	a	3.85*	2.88	+
	Xgwm146	d	15.45***	10.62	+
	Xcfa2171	c'	4.39*	3.29	-
	Xcfa2171	f	5.32*	3.96	+
SDS[i]	Xgwm550	a	5.75*	4.30	+
	Xgwm550	c	9.80**	7.11	-
	Xgwm499	d	6.29*	4.62	-
	Xcfa2171	d	3.88*	2.92	+
GPC[j]	Xgwm550	d	16.44***	12.60	+
	Xwmc522	a'	12.28**	9.65	+
	Xgwm299	a	4.41*	3.73	+
	Xgwm46	d	8.91**	7.31	+
	Xgwm499	a	21.77***	15.92	+
	Xgwm499	b	8.72**	7.05	-
	Xgwm344	a	7.47**	6.10	+
	Xgwm146	a	3.81*	3.21	-
	Xgwm146	d	9.07**	7.31	+
	Xcfa2171	f	15.96**	12.37	+

[a] F-values;* for $P < 0.05$, ** for $P < 0.01$, and $P < 0.001$*** ; [c]- negativeeffect, + positiveeffect.
[b] % of variation of a trait explained by a marker out of total significantly associated markers; [d] 1000-kernel weight; [e] test weight; [f] yellow pigment content; [g] yellow index brightness; [h] SDS-volume sedimentation; [i] grain protein content.

Allelic variation of SSR[1] *loci that control quality related traits*

Using Fisher's least significant difference (LSD) method, it was possible to identify a significant difference in allele effects across durum wheat accessions (Table 4). Sixteen allele markers were significantly associated with TKW. Of these, *Xgwm371b*, *Xgwm550a*, *Xwmc522c*, *Xgwm299b*, *Xgwm344b*, and *Xgwm146b* were significantly positively associated with 1000-kernel weight, indicating that the alleles increased 1000-kernel weight's phenotypic value. On the other hand, *Xgwm371a*, *Xgwm550d*, *Xwmc522a*, *Xwmc522b*, *Xgwm299a*, *Xgwm46a*, *Xgwm499a*, *Xgwm344c*, *Xgwm344d*, and *Xcfa2174f* had a negative allele effect. Twenty alleles were significantly associated with TW. Of these, *Xgwm471a*, *Xgwm371b*, *Xgwm550a*, *Xwmc522c*, *Xgwm299b*, *Xgwm499b*, *Xgwm146b*, and *Xcfa2174c* were positively associated with TW, indicating that these alleles increased the phenotypic value of TW. *Xgwm471b*, *Xgwm371a*, *Xgwm550d*, *Xgwm 550c*, *Xwmc522a'*, *Xgwm299a*, *Xgwm499a*, *Xgwm344a*, *Xg wm146d*, *Xcfa2174a*, *Xcfa2174b*, and *Xcfa2174f* were relatively common alleles, although allele distribution was uneven in the seven investigated traits. Eleven common alleles were shared between TW and TKW; of these, *Xgwm371a*, *Xgwm299a*, *Xgwm499a*, and *Xcfa2174f* showed a large and significant positive allele effect, indicating that the alleles increased the phenotypic value of the related milling traits. *Xgwm371b*, *Xgwm550a*, *Xwmc522c*, *Xgwm299b*, and *Xgwm146b* exhibited a negative allele effect. With respect to grain protein content, ten SSR[1] alleles were identified. Of these, eight alleles (*Xgwm550d*, *Xwmc522a'*, Xgwm299a, *Xgwm46d*, *Xgwm499a*, *Xgwm344a*, *Xcfa2171f*, and *Xgwm146d*) had a positive effect (Table 4), indicating that the alleles increased the phenotypic value of the GPC. Two alleles, *Xgwm499b* and *Xgwm146d*, had a negative effect. Similarly, for SDS-sedimentation volume, four SSR[1] marker alleles were detected. Of these, *Xgwm550a* and *Xcfa2174d* had a significant positive allele effect, indicating that the alleles increased the phenotypic value of SDS-sedimentation volume. *Xgwm550c* and *Xgwm499d* had a negative allele effect. Thirteen SSR[1] markers were detected for YP. Of these, *Xgwm550d*, *Xwmc522a'*, *Xgwm299a*, *Xgwm499a*, *Xgwm344a*, *Xgwm408a*, *Xgwm146d*, and *Xcfa2174f* showed a large positive allele effect, indicating that the alleles increased the phenotypic value of the YP trait; *Xwmc283a*, *Xwmc283e*, *Xgwm299b*, *Xgwm344b*, and *Xcfa2174c'* showed a negative allele effect. Similarly, 10 allele markers were detected for b[5], of which *Xgwm508b*, *Xgwm299a*, *Xgwm 344e*, *Xgwm408a*, and *Xgwm146d* showed a large positive allele effect and *Xgwm508a*, *Xwmc283e*, *Xgwm299b*, *Xgwm344b*, *Xgwm408c*, and *Xgwm146b* showed a negative allele effect. Four allele markers were identified with respect to L[4], with *Xgwm46a* and *Xgwm344d* showing a large positive allele effect and *Xgwm408b* and *XdupW38b* showing a negative allele effect. In total, six alleles derived from the five SSR[1] loci were shared between the yellow pigment-related traits. Three (*Xwmc283e*, *Xgwm299b*, and *Xgwm 344b*) had a high positive allele effect, and *Xgwm299a*, *Xgwm408a*, *Xgwm146d* had a negative allele effect.

Discussion

To maintain relatively high polymorphism levels and to take advantage of association mapping, different germplasm from the durum wheat breeding program of the National Institute of Agronomical Research of Rabat, Morocco, including Mediterranean landraces, advanced lines from CYMMIT[2] and ICARDA[3], and Moroccan cultivars, were used in the panel.

Genetic diversity was high within populations or germplasm types but relatively low between populations. These findings are in accordance with a number of studies (Arora et al. 2014; Dashchi et al. 2012; Mahjoub et al. 2012). The low variability among populations can be explained by the high gene flow value obtained, which is a general indicator of the magnitude of genetic exchange (Abouzied et al. 2013), the level of differentiation among the population being inversely proportional to the value of gene flow.

Quality traits analysis

Positive changes in yield-related traits over time were due to substantial increases in TKW and TW due to an increase in selection pressure compensating for the significant decrease in GPC. The negative relationship between yield and protein content (Rharrabti et al. 2001) has been associated with a dilution effect of nitrogen compounds when carbohydrate deposition increases via photosynthesis (Lawlor 2002; Martre et al. 2003). Nevertheless, this decrease in protein content was relative and did not result in values below 14% in any of the durum wheat groups considered, a level that exceeds the minimum values required by the pasta processing industry (set at ~12.5 %; Peña et al. 2002). The largest quality trait improvements from landraces to advanced lines occurred in gluten strength, but this increase partially failed in the Moroccan varieties. Both the YP content and yellow index traits suffered a significant decrease in Moroccan cultivars and international lines compared to the Mediterranean and Moroccan landraces. Since this trait is largely controlled by additive gene effects with generally high heritability (Blanco et al. 2011; Elouafi et al. 2001; Taghouti et al. 2010), it will be useful to exploit landrace richness in breeding programs aiming to improve the nutritional value of durum and end-products.

SSR[1] marker quality trait associations

Individual SSR[1] markers were associated with two to six quality traits. These co-localized, and pleiotropic associations may be beneficial for detecting important genomic regions or genes for quality traits. Furthermore, markers associated with more than one trait may be useful for improving more than one

trait by marker-assisted selection (Sun et al. 2015). Only three SSRs[1] (*Xgwm508*, *Xgwm471*, and *XdupW38*) were associated with only one trait (Table 4). Our results agree with other studies in wheat for the expressed sequence tag SSR[1] *XdupW38* (Eujayl et al. 2002), in which the overall level of polymorphisms in genomic SSRs[1] was higher than that for expressed sequence tag SSRs[1], despite being abundant in transcribed regions (Morgante et al. 2002). With respect to markers expected to be associated with traits (*Xgwm371* and *Xgwm508*) in previous studies, here they were associated with different traits. Yu et al. (2011) reported that there is no guarantee that molecular markers identified in one population will be useful in other populations when the populations originate from distantly related germplasms. For example, Parker et al. (1998) identified a wheat flour color marker on chromosome 7A based on a Schomburgk /Yaralinka cross and later used it in the Cranbrook/Halberd and Sunco/Tasman crosses (Mares and Campbell 2001). The same marker was not applicable to the yellow color characteristics of Cunningham and Janz lines, but it was applicable to material with Schomburgk-type yellow flour color (Sharp et al. 2001). Furthermore, previous studies have reported that quantitative trait loci expression can be influenced by internal and external factors such as the environment, genotype, developmental stage, and related traits. Such quantitative trait loci are unstable and specific and may be suitable only in specific environments and at certain developmental stages, or may only be suitable in one population for marker-assisted selection (Wang et al. 2012). The putative marker alleles identified in this study and those from previous studies (Table S3) are likely to be important in breeding programs and should be considered for use in marker-assisted selection programs. Based on correlation and effect estimation analyses, most of the SSR[1] markers reported by other authors (Table S3) in association with YP were validated, i.e., *Xgwm344* and *Xgwm146* loci on chromosome 7BL, *Xgwm408* on 5BL, *Xgwm299* on 2BS, and *Xwmc283* on 7A (Blanco et al. 2011; Elouafi et al. 2001; Howitt et al. 2009; Kuchel et al. 2006; Mares and Campbell 2001; Patil et al. 2008a; Reimer et al. 2008; Zhang and Dubcovsky 2008; Zhang et al. 2008); these are potential new loci for YP. Thus, the combination of these five markers may improve the accuracy of marker-assisted selection for the YP trait in durum wheat.

Allelic variation of loci that control quality-related traits

Alleles were relatively common, although we note that the distribution of the alleles was uneven in the seven investigated traits. One thousand-kernel weight and test weight are important for grain quality and yield in durum wheat; therefore, these traits have drawn major attention from the global wheat breeding community (Patil et al. 2008b). There were 11 relatively common alleles significantly associated with TW and TKW, five of which (*Xgwm371b*, *Xgwm299b*, *Xgwm499b*, *Xgwm550a*, and *Xcfa2174c*) had a large and significant positive allele effect indicating that the alleles increased the phenotypic value of the related milling traits and explained phenotypic variation. This confirms the positive and strong correlation between TW and TKW as measured by the Pearson's coefficient correlation. Furthermore, Elouafi et al. 2001 estimates of TW and TKW heritability in the broad sense were relatively intermediate to high. This indicates the association is genetic in nature and suggested that selection will be valuable in early generations. These will be useful for molecular marker assisted selection and molecular design breeding. Grain protein content partially determines the nutritional value and baking properties of common wheat and, together with GPC and SDS-sedimentation volume, are the most important factors affecting pasta characteristics. In general, high grain protein content is associated with pasta firmness and greater tolerance to over-cooking, and semolina protein concentration alone accounts for 30-40% of the variability in pasta cooking quality (Dexter and Matsuo 1977). With respect to the allelic variants, 10 SSR[1] marker alleles were significantly associated with GPC (Table 6). These marker loci explained 3.81-21.77% of the total variation. By comparing the allele effects associated with TW, TKW, and GPC, we found that most of the alleles positively associated with GPC (*Xgwm550d*, *Xwmc522a'*, *Xgwm299a*, *Xgwm499a*, and *Xgwm344a*) had a significant negative effect on TW and TKW values. *Xgwm499b* had a negative effect on GPC but was positively associated with TW and TKW. This is expected, since an inverse correlation between GPC and yield-related traits was detected by the Pearson's correlation coefficient (Table 2) and which is in agreement with the finding of previous authors (Blanco et al. 2002).

With respect to gluten strength, four SSR[1] markers were significantly associated with SDS-sedimentation volume (Table 4). Of these, *Xgwm550c* and *Xgwm499d* had a significant positive allele effect, indicating that the alleles increased the phenotypic value of SDS-sedimentation volume. Moreover, Elouafi et al. (2001) and Taghouti et al. (2010) indicate a strong genotypic effects on this traits with a high heritability value (0.75), suggesting to use these alleles markers as a direct criterion for improving gluten strength of durum wheat.

Flour color is important for flour quality and exerts a significant influence on products such as noodles (Parker et al. 1998). Nine SSR[1] marker alleles were significantly associated with YP content (Table 4). Of these, *Xgwm146d* was most strongly associated, followed by *Xwmc283e*, *Xgwm344b*, and *Xgwm550d*. In total, six alleles derived from the five aforementioned SSR[1] loci were shared between the yellow pigment-related traits. Three of these, *Xwmc283e*, *Xgwm299b*, and *Xgwm344b*, showed high positive allele effects, indicating that the alleles increased the phenotypic value of the YP trait. These common alleles should be used in breeding programs as marker-assisted selection for quality traits.

As expected, correlated traits shared common genomic regions; this was especially apparent for associations between TW and TKW and YP concentration with one of its compo-

nent traits (yellow index b) with high heritability in broad sense. The results suggest that simultaneous improvement of TW and TKW, as well as yellow color components, could be achieved in durum wheat breeding since there some genomic regions (and specific marker alleles) are common to these traits. Indeed, Sun et al. (2015) reported that when most marker alleles are associated with the same trait, the marker may be linked with a crucial gene necessary for trait regulation.

Conclusions

Significant associations between quality-related traits and SSR[1] markers were found in the collection. The combined use of molecular markers and quality trait data illustrated how markers can usefully be used to detect appropriate alleles in different genetic backgrounds for selecting genotypes of interest in marker-assisted selection improvement programs. A number of promising SSR marker alleles associated with quality traits, namely *Xgwm344b* and *Xgwm146d*, *Xgwm408a*, *Xgwm299b*, and *Xgwm283e* for YP and *Xgwm371b*, *Xgwm299b*, *Xgwm499b*, *Xgwm550a* and *Xcfa2174c* for semolina yield traits (TW and TKW), may be particularly useful since they were also validated in previous studies. These are, therefore, attractive candidates for immediate use by durum wheat breeders. However, some markers associated with traits in this study were associated with other traits in previous studies. These need to be validated using further different durum wheat populations in different environments.

The alleles identified in this study provide an opportunity to develop recurrent marker-assisted selection, thus increasing the alleles insignificantly associated genomic regions. Furthermore, these data are useful for effectively exploiting genetic variations in landraces in durum wheat breeding with marker-assisted selection programs.

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References

AACC. 1984. Approved Methods 7th edition, St Paul, Minnesota. USA

Abouzied HM, Eldemery SMM, Abdellatif KF. 2013. SSR-based genetic diversity assessement in tetraploid and hexaploid wheat populations. Br. Biotechnol J. 3: 390-404

Arora A, Kundu S, Dilbaghi N, Sharma I, Tiwari R. 2014. Population structure and genetic diversity among Indian wheat varieties using microsatellite (SSR) markers. Amer. J. Crop Sci. 8: 1281-1289

Benbouza H, Jacquemin JM, Baudoin JP, Mergeai G. 2006. Optimization of a reliable, fast, cheap and sensitive silver staining method to detect SSR markers in polyacrylamide gels. Biotechnol. Agron. Soc. Environ. 10: 77-81

Blanco A, Colasuonno P, Gadaleta A, Mangini G, Schiavulli A, Simeone R, Digesù AM, De Vita P, Mastrangelo AM, Cattivelli L. 2011. Quantitative trait loci for yellow pigment concentration and individual carotenoid compounds in durum wheat. J. Cereal Sci. 54: 255-264

Blanco A, Pasqualone A, Troccoli A, Di Fonzo N, Simeone R. 2002. Detection of grain protein content QTLs across environments in tetraploid wheats. Plant Mol. Biol. 48: 615-623

Blanco A, Simeone R, Gadaleta A. 2006. Detection of QTLs for grain protein content in durum wheat. Theor. Appl. Genet. 112: 1195-1204

Caballero L, Martin LM, Alvarez JB. 2008. Relationships between the HMW- and LMW-glutenin subunits and SDS-sedimentation volume in Spanish hulled wheat lines. Czech J. Genet. Plant Breed. 44: 114-117

Dashchi S, Abdollahi Mandoulakani B, Darvishzade R, Bernousi I. 2012. Molecular similarity relationships among Iranian bread wheat cultivars and breeding lines using ISSR markers. Not. Bot. Horti. Agrobo. 40: 254-260

De Vienne D, Causse M. 1998. La cartographie et la caractérisation des locus contrôlant la variation des caractères quantitatifs. In D De Vienne, ed, Les marqueurs moléculaires en génétique et biotechnologie végétales, INRA, Ed. 2, Paris pp 89-118

Dexter JE, Matsuo RR. 1977. Influence of protein content on some durum wheat quality parameters. Can. J. Plant Sci. 57: 717-727

Distelfeld A, Uauy C, Fahima T, Dubcovsky J. 2006. Physical map of the wheat high-grain protein content gene Gpc-B1 and development of a high-throughput molecular marker. New Phytol. 169: 753-763

Duveiller E, Singh RP, Nicol JM. 2007. The challenges of maintaining wheat productivity: pests, diseases and potential epidemics. Euphytica 157: 417-430

Elouafi I. 2003. Quantitative Trait Loci (QTL) determination of grain quality traits in durum wheat (*Triticum turgidum L. var. durum*). Ph. D. thesis, Cordoba University, Cordoba

Elouafi I, Nachit NM, Martin LM. 2001. Identification of a microsatellite on chromosome 7B showing a strong linkage with yellow pigment in durum wheat *(Triticum turgidum L. var. durum).* Hereditas 135: 255-261

Eujayl I, Sorrells ME, Baum M, Wolters P, Powell W. 2002. Isolation of EST-derived microsatellite markers for genotyping the A and B genomes of wheat. Theor. Appl. Genet. 104: 399-407

Flint-Garcia SA, Thuillet AC, Yu J, Pressoir G, Romero SM, Mitchell SE, Doebley J, Kresovich S, Goodman MM,

Buckler ES. 2005. Maize association population: a high-resolution platform for quantitative trait locus dissection. Plant J. 44: 1054-1064

Goutam U, Kukreja S, Tiwari R, Chaudhury A, Gupta RK, Dholakia BB, Yadav R. 2013. Biotechnological approaches for grain quality improvement in wheat: Present status and future possibilities. Aust. J. Crop Sc. 7: 469-483

Gupta PK, Balyan HS, Edwards KJ, Isaac P, Korzun V, et al. 2002. Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theor. Appl. Genet. 105: 413-422

Gustafson JP, Maccaferri M, Stefanelli S, Rotondo F, Tuberosa R, Sanguineti MC. 2007. Relationships among durum wheat accessions. I. Comparative analysis of SSR, AFLP and phenotypic data. Genome 50: 373-384

Guyomarc'h H, Sourdille P, Charmet G, Edwards K, Bernard M. 2002. Characterization of polymorphic microsatellite markers from Aegilops tauschii and transferability to the D-genome of bread wheat. Theor. Appl. Genet. 104: 1164-1172

Haussmann BIG, Parzies HK, Presterl T, Suπi? Z and Miedaner T. 2004. Plant genetic resources in crop improvement. Plant Genetic Res. 2: 3-21

Howitt CA, Cavanagh CR, Bowerman AF, Cazzonelli C, Rampling L, Mimica JL, Pogson BJ. 2009. Alternative splicing, activation of cryptic exons and amino acid substitutions in carotenoid biosynthetic genes are associated with lutein accumulation in wheat endosperm. Funct. Integr. Genomics 9: 363-376

Howitt CA, Gale KR, Juhasz A. 2007. Diagnostic markers for quality. In Wringley C, Bekes F, Bushuk W, Gliadin and Glutenin: The unique balance of wheat quality, BUSHUK eds, AACC International, USA

Jing HC, Kornyukhin D, Kanyuka K, Orford S, Zlatska A, Mitrofanova OP, Koebner R, Hammond-Kosack K. 2007. Identification of variation in adaptively important traits and genome-wide analysis of trait-marker associations in *Triticum monococcum*. J. Exp. Bot. 58: 3749-3764

Kerfal S, Giraldo P, Rodriguez-Quijano M, Vazquez JF, Adams K, Lukow OM, Roder MS, Somers DJ, Carrillo JM. 2010. Mapping quantitative trait loci (QTLs) associated with dough quality in a soft x hard bread wheat progeny. J. Cereal Sci. 52: 46-52

Kuchel H, Langridge P, Mosionek L, Williams K, Jefferies SP. 2006. The genetic control of milling yield, dough rheology and baking quality of wheat. Theor. Appl. Genet. 112: 1487-1495

Lawlor DW. 2002. Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. J. Exp. Bot. 53: 773-787

Maccaferri M, Sanguineti MC, Donini P, Tuberosa R. 2003. Microsatellite analysis reveals a progressive widening of the genetic basis in the elite durum wheat germplasm. Theor. Appl. Genet. 107: 783-797

Maccaferri M, Sanguineti MC, Natoli E, Araus-Ortega JL, Ben Salem M, et al. 2006. A panel of elite accessions of durum wheat (*Triticum durum Desf.*) suitable for association mapping studies. Plant Genet. Res. 4: 79-85

Maccaferri M, Sanguineti MC, Mantovani P. Demontis A, Massi A, Ammar K. Kolmer JA, Czembor JH, Ezrati S, Tuberosa R. 2010. Association mapping of leaf rust response in durum wheat. Mol. Breed. 26: 189-228

Maccaferri M, Sanguineti MC, Garcia Del Moral L, Demontis A, El-Ahmed A, et al. 2011. Association mapping in durum wheat grown across a broad range of water regimes and yield potential. J. Exp. Bot. 62: 409-438

Mahjoub AA, Mguis K, Rouaissi M, Abdellaoui R, Brahim NB. 2012. RAPD analysis of genetic diversity in natural populations of Aegilops geniculata Roth and *Triticum durum Desf* from Tunisia. Agric. Biol. J. N. Am. 3: 466-475

Mares DJ, Campbell AW. 2001. Mapping components of flour and noodle colour in Australian wheat. Aust. J. Agr. Res. 52: 1297-1309

Martre P, Porter JR, Jamieson PD, Triboï E. 2003. Modeling grain nitrogen accumulation and protein composition to understand the sink/source regulations of nitrogen remobilization for wheat. Plant Physiol. 133: 1959-1967

Morgante M, Hanafey M, Powell W. 2002. Microsatellites are preferentially associated with non repetitive DNA in plant genomes. Nat Genet 30:194-200

Morgante M, Olivieri AM, 1993. PCR-amplified microsatellites as markers in plant genetics. Plant J. 3: 175-82.

Olmos S, Distelfeld A, Chicaiza O, Schlatter AR, Fahima T, Echenique V, Dubcovsky J. 2003. Precise mapping of a locus affecting grain protein content in durum wheat. Theor. Appl. Genet. 107: 1243-1251

Parker GD, Chalmers KJ, Rathjen AJ, Langridge P. 1998. Mapping loci associated with flour color in wheat. Theor. Appl. Genet. 97: 238-245

Patil RM, Oak MD, Tamhankar SA, Sourdille P, Rao VS. 2008a. Mapping and validation of a major QTL for yellow pigment content on 7AL in durum wheat (*Triticum turgidum L. ssp. durum).* Mol. Breeding 21: 485-496

Patil RM, Oak MD, Tamhankar SA, Rao VS. 2008b. Mapping QTLs for quality characters in durum wheat (*Triticum turgidum L. ssp. durum*). Sydney University Press, pp 1-3

Peña RJ, Trethowan R, Pfeiffer WH, Van Ginkel M. 2002. Quality (end-use) improvement in wheat: Compositional, genetic, and environmental factors. J. Crop Prod. 5: 1-37

Ramya P., Chaubal A., Kulkarni K., Gupta L., Kadoo N., Dhaliwal H. S., Chhuneja P., Lagu M., Gupta V. 2010. QTL mapping of 1000-kernel weight, kernel length, and kernel width in bread wheat (*Triticum aestivum L.*) J Appl Genet 51: 421–429

Reimer SO. 2008. Association mapping of endosperm colour in durum wheat (*Triticum turgidum L. var. durum*). Ph. D. Thesis. University of Saskatchewan Saskatoon, Saskatchewan

Rharrabti Y, Villegas D, García del Moral LF, Aparicio N, Elhani S, Royo C. 2001. Environmental and genetic determination of protein content and grain yield in durum

wheat under Mediterranean conditions. Plant Breeding 120: 381–388.
Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW. 1998. A microsatellite map of wheat. Genetics 149: 2007-2023
Roy JK, Bandopadhyay R, Rustgi S, Balyan HS, Gupta PK. 2006. Association analysis of agro-morphologically important traits using SSR, SAMPL and AFLP markers in bread wheat. Curr. Sci. India 90: 683-389
Santra M, Rao VS, Tamhankar SA. 2003. Modification of AACC procedure for measuring ?-Carotene in early generation durum wheat. Cereal Chem. J. 80: 130-131
Sharp PJ, Johnston S, Brown G, Mcintosh RA, Pallotta M, et al. 2001. Validation of molecular markers for wheat breeding. Crop Pasture Sci. 52: 1357-1366
Singh R, Hucl P, Båga M, Chibbar RN. 2012. Validation of molecular markers for pre-harvest sprouting resistance in bread wheat. Cereal Res. Commun. 40: 194-203
Singh A., Reimer S., Pozniak C.J., Clarke F.R., Clarke J.M., Knox R.E., Singh A.K. 2009. Allelic variation at Psy1-A1 and association with yellow pigment in durum wheat grain. Theor. Appl. Genet. 118 : 1539-1548.
Sourdille P, Cadalen T, Guyomarc'h H, Snape JW, Perretant MR, Charmet G, Boeuf C, Bernard S, Bernard M. 2003. An update of the Courtot ˇ Chinese Spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. Theor. Appl. Genet. 106: 530–538
Sun X, Du Z, Ren J, Amombo E, Hu T, Fu J. 2015. Association of SSR markers with functional traits from heat stress in diverse tall fescue accessions. BMC Plant Biology 15:116
Taghouti M, Gaboun F, Nsarellah N, Rhrib R, El-Haila M, Kamar M, Abbad-Andaloussi F, Udupa SM. 2010. Genotype x Environment interaction for quality traits in durum wheat cultivars adapted to different environments. Afr. J. Biotechnol. 9: 3054-3062
Tang JC, Camberato JJ, Yu XQ, Luo N, Bian SM, Jiang YW. 2013. Growth response carbohydrate and ion accumulation of diverse perennial ryegrass accessions to increasing salinity. Sci. Hortic. 154: 73-81
Terracciano I, Maccaferri M, Bassi F, Mantovani P, Sanguineti MC. 2013. Development of COS-SNP and HRM markers for high throughput and reliable haplotype-based detection of Lr14a in durum wheat (*Triticum durum Desf.*). Theor. Appl. Genet. 126: 1077-1101
Wang L, Cui F, Wang J, Jun L, Ding A, Zhao C, Li X, Feng D, Gao J, Wang H. 2012. Conditional QTL mapping of protein content in wheat with respect to grain yield and its components. J. Genet. 91: 303-312
Winter P, Kahl G. 1995. Molecular marker technologies for plant improvement. World J. Microb. Biot. 11: 438-448
Yu X, Bai G, Luo N, Chen Z, Liu S, Liu J, Warnke SE, Liu J, Warnke SE, Jiang Y. 2011. Association of simple sequence repeat (SSR) markers with submergence tolerance in diverse populations of perennial ryegrass. Plant Sci. 180: 391-398
Zhang W, Chao S, Manthey F, Chicaiza O, Brevis JC, Echenique V, Dubcovsky J. 2008. QTL analysis of pasta quality using a composite microsatellite and SNP map of durum wheat. Theor. Appl. Genet. 117: 1361-1377
Zhang W, Dubcovsky J. 2008. Association between allelic variation at the Phytoene synthase gene and yellow pigment content in the wheat grain. Theor. Appl. Genet. 116: 635-645

Footnotes
[1] Simple Sequence Repeat
[2] International Maize and Wheat Improvement Center
[3] International Center for Agricultural Research in the Dry Areas
[4] Brightness
[5] Yellow Index
[6] Polymerase Chain Reaction

Table S1. Origine and pedigree of the 132 accessions used in the experiment

Code	Type	IG/Pedigree
1	IALC	Stinkpot//Altar-84/Alondra
2	IALC	Guanay/3/Stot//Altar 84/Ald
3	IALC	Cbc 509 Chile/Somat_3.1//Woduck/Cham_3
4	IALC	Sora/2*Plata_12//Rascon_37/4/Yazi_1/Akaki_4//Somat_3/3/Auk/Guil//Green
5	IALC	Rissa/Gan//Poho_1/3/Plata_3//Crex/Alla/4/Jupare C 2001/5/Arment//Srn_3/Nigris_4/3/Canelo_9.1
6	IALC	Cbc 509 Chile//2*Tilo_1/Lotus_4
7	IALC	Somat_3/3/Stot//Altar 84/Ald/4/Wizza_23/Cona-D
8	IALC	Aaz/Morus_1//Rcol/3/Somat_3/Phax_1//Tilo_1/Lotus_4
9	IALC	Rissa/Gan//Poho_1/3/Plata_3//Crex/Alla/7/Eudo//Chen_1/Tez/3/Tantlo_1/5/Chen/Altar 84/3/Hui/Poc//Bub/
10	IALC	Kucuk_2/Pata_2/4/Arment//Srn_3/Nigris_4/3/Canelo_9.1
11	IALC	Topdy_18/Focha_1//Altar 84/3/Ajaia_12/F3local(Sel.Ethio.135.85)//Plata_13/4/Somat_3/Green_22
12	IALC	Toska_26/Rascon_37//Snitan/4/Arment//Srn_3/Nigris_4/3/Canelo_9.1
13	IALC	1A.1D 5+1-06/3*Mojo//Rcol/4/Arment//Srn_3/Nigris_4/3/Canelo_9.1
14	IALC	Sooty_9/Rascon_37//Woduck/Cham_3/10/Plata_10/6/Mque/4/Usda573//Qfn/Aa_7/3/Alba-D/5/Avo/Hui/7/Plata_1
15	IALC	Cbc 514 Chile/Somat_4/3/Ajaia_12/F3local(Sel.Ethio.135.85)//Plata_13/6/Chen/Altar 84/3/Hui/Poc//Bub/
16	IALC	Arment//2*Sooty_9/Rascon_37/4/Cndo/Primadur//Hai-Ou_17/3/Snitan
17	IALC	Lotus_5/Sord_1/3/Tatler_1/Solga_5//Pon_2/4/Arment//Srn_3/Nigris_4/3/Canelo_9.1
18	IALC	Rcol/Poho_1/3/Dipper_2/Bushen_3//Snitan
19	IALC	Dipper_2/Bushen_3//Snitan/3/Somat_3/Phax_1//Tilo_1/Lotus_4/5/Patin_7//Hui/Yav79/3/Ajaia_12/F3local(S
20	MV	Jo"S"/Aa"S"//Fg"S"
21	IALC	Sooty_9/Rascon_37//Storlom
22	IALI	Ammar-6
23	IALI	Unknown
24	IALI	Geruftel-1
25	IALC	Eth-Lrbr-A-1-133/3*Altar-84/Jupare-C 2001
26	IALI	Mrb3/Mna1//Ter1
27	IALI	Azeghar-1/3/Mrf2//Bcr/Gro1
28	IALC	Dipper_2/Bushen_3//Snitan
29	IALI	Azeghar2//Ossl1/Stj5
30	IALI	Icamor-Ta04-59
31	IALC	Storlom/3/Rascon_37/Tarro_2//Rascon_37
32	IALC	Shag_14/Anade_1//Kitti_1
33	IALC	Hadj-Mouline/Sabil-2/Canelo_9.1
34	IALI	unknown
35	IALI	Ossl-1/4/MrbSH/3/Rabi//Gs/Cr/5/Chan
36	IALC	Cs/Th.Cu//Glen/3/Gen/4/Myna/Vul/5/2*Don87/6/2*Busca_3
37	IALC	Plata_10/6/Mque/4/Usda573//Qfn/Aa_7/3/Alba-D/5/Avo/Hui/7/Plata_13/8/Thknee_11/9/Chen/Altar 84/3/Hui/Poc//Bub/Rufo/4/Fnfoot
38	IALC	Dukem_1//Patka_7/Yazi_1/3/Patka_7/Yazi_1
39	IALC	Guayacan Inia/3/Stot//Altar 84/Ald
40	IALC	Cbc 509 Chile/3/Auk/Guil//Green
41	IALI	Bicrederaa-1//Saadi 1989/Chan
42	IALC	Altar 84/Stint//Silver_45/3/Guanay/4/Green_14//Yav_10/Auk
43	IALC	Altar 84/Stint//Silver_45/4/Skest//Hui/Tub/3/Silver/5/Ajaia_12/F3local(Sel.Ethio.135.85)//Plata_13
44	IALC	Arlin/2*Aco89//Jupare C 2001
45	IALC	Stot//Altar 84/Ald/3/Snitan
46	IALC	Stot//Altar 84/Ald/3/Thb/Cep7780//2*Musk_4/4/Auk/Guil//Green
47	IALI	Bcrch1/DCD DW 7//Ossl-1/Gdfl
48	IALI	Azeghar-1
49	IALI	Ammar-8
50	IALI	Geruftel-1
51	IALI	Icajihan4
52	IALI	Icajihan26
53	IALI	Ossl1/Stj5/5/Bicrederaa1/4/Bezaiz-SHF//SD-19539/Waha/3/Stj/Mrb3
54	IALI	Korifla (Check)
55	IALI	Magh72/Rufo//Alg86/Ru/3/Altar 84/Ald/4/../5/Msbl-1/Quarmal
56	IALC	Ajaia_16//Hora/Jro/3/Gan/4/Zar/5/Suok_7/6/Jupare C 2001
57	IALC	Rascon_21/3/Mque/Alo//Foja/4/Guanay/5/Topdy_18/Focha_1//Altar 84
58	IALC	Ruff/Flamingo,Mex//Mexicali-75/3/Shearwater
59	IALC	Cbc 509 Chile/Yebas_8//Dukem_12/2*Rascon_21
60	IALC	Stinkpot//Altar-84/Alondra
61	IALC	Stinkpot//Altar-84/Alondra*2/3/Auk/Guil//Green
62	IALC	Cbc 514 Chile/Somat_4/3/Ajaia_12/F3local(Sel.Ethio.135.85)//Plata_13
63	IALC	Cndo/Vee//7*Plata_8/3/Topdy_18/Focha_1/4/Jupare C 2001
64	IALC	Srn_1/6/Fgo/Dom//Nach/5/Altar 84/4/Garza/Afn//Cra/3/Gerardo Vz 394/7/Ld357e/2*Tc60//Jo69/3/Fgo/4/Gta/5/Cndo/8/Green_38/9/2*Jupare C 2001
65	IALC	Poho_1//Mojo/Kitti/3/Pod_11/Yazi_1
66	Cocorit	Rae/4*Tc60//Stw63/3/Aa's'=Cisnne
67	2909	SEL In Old Moroccan Population
68	Kypernda	Inra. Selection In CyprusPopulaion
69	Jori	Bye*2/Tc60//Tac125e/3*Tc60
70	Marzak	INRA EII.12 Selection In CIMMYT
71	Karim	Jo"S"/Aa"S"//Fg"S"

Code	Type	IG/Pedigree
72	Acsad65	Gdovz469/3/Jo"S"//61.130/Lds
73	Belbachir	Gdovz469/3/Jo//61.130/Lds
74	O. Rabiaa	Hau/Jori69
75	Sarif	Lds/Mut//Teal's'
76	Sebou	Cr/Pol (Grebe's')
77	Anwar	Anouar : 1749. Inra Bdv2 90
78	Amjad	T.Turgidum/3/Aa/Cr/Cit//Bit
79	Marjana	D63.3/Gta/4/Ato/2/Aa/Ple/3/Dl67.2/Swan//
80	Jawhar	Inra 1750 Inra Bdy8 90
81	Tomouh	Oum Rabia #6
82	Nassira	Ta14/Bd3//Isly # Cf41530-1548 #
83	Chaoui	Karim / Cocorit // Rsmor2bc1f1 /3/ Mzk # Cf
84	Marwan	Sebou / Bt40 // Sarif #Cf4(1896-1904)
85	Amria	H.Mouline / Saada // Karim #Cf4
86	MEDL	IG 43905/LBY
87	MEDL	IG 83078/EGY
88	MEDL	IG 93811/DZA
89	MEDL	IG 94062/TUN
90	MEDL	IG 94817TUN
91	MEDL	IG 95842/SYR
92	MEDL	IG 95872/SYR
93	MEDL	IG 96186/JOR
94	MEDL	IG 97385/DZA
95	MEDL	IG 98022/EGY
96	MEDL	IG 98023/EGY
97	MEDL	IG 98028/EGY
98	MEDL	IG98135/LBY
99	ML	IG98721/MAR
100	MEDL	IG98725/LBY
101	MEDL	IG115812/JOR
102	MEDL	IG127163/PAL
103	ML	MAR P35
104	ML	MAR P46
105	ML	MAR P81
106	ML	MAR P109
107	ML	MAR P110
108	ML	MAR P112
109	ML	MAR P 113
110	ML	MAR P114
111	ML	MAR P115
112	ML	MAR P3/2
113	ML	MAR P5/3
114	ML	MAR P5/14
115	ML	MAR P5/16
116	ML	MAR P5/20
117	ML	MAR P5/23
118	ML	MAR P8/9
119	ML	MAR P10/10
120	ML	MAR P10/32
121	ML	MAR P10/34
122	ML	MAR P10/46
123	ML	MAR P10/49
124	ML	MAR P105/14
125	ML	MAR P105/19
126	ML	Atsiki-3
127	ML	Menceki
128	ML	Korifla
129	ML	ICAHEFR-ICD95-0638-1=HF ICD95-0638-T-0AP-3AP-0AP-4AP-0TR-3AP-AP-2AP-0AI
130	ML	Gidara-2
131	ML	MAR P7
132	ML	MAR P117

MEDL: Mediterranean landraces; ML:Moroccan landraces; IALC: CYMMIT international lines; [k] ICARDA: international lines; accession number from 66 to 85 are Moroccan varieties;the Moroccanlandracesnumberfrom 105 to 125 are collectedfromdifferents sites in Morocco and stocked at the National Institute of Morocco in Rabat.

Table S2. Probed microsatellites sequence and their optimal annealing temperatures

Marker	Sequence (5' -3')	Ch	L	Repeated Motif	T(°C)
Xgwm46	GCACGTGAATGGATTGGAC	7BS	20	$(GA)_2GC(GA)_{33}$	55.3
	TGACCCAATAGTGGTGGTCA		20		
Xgwm146	CCAAAAAAACTGCCTGCATG	7BL	20	$(GA)_5$ GG$(GA)_{20}$	55.35
	CTCTGGCATTGCTCCTTGG		19		
Xgwm299	ACTACTTAGGCCTCCCGCC	2BS	19	$(GA)_{31}$ $(TAG)_4$	56.45
	TGACCCACTTGCAATTCATC		20		
Xgwm344	CAAGGAAATAGGCGGTAACT	7BL	20	$(GT)_{24}$	51.35
	ATTTGAGTCTGAAGTTTGCA		20		
Xgwm371	GACCAAGATATTCAAACTGGCC	5BL	22	$(CA)_{10}(GA)_{32}$	56.65
	AGCTCAGCTTGCTTGGTACC		20		
Xgwm408	TCGATTTATTTGGGCCACTG	5BL	20	$CA)>_{22}(TA)(CA)_7$	54.5
	GTATAATTCGTTCACAGCACGC		22		
Xgwm471	CGGCCCTATCATGGCTG	7AS	17	$(TA)_9$	55.2
	GCTTGCAAGTTCCATTTTGC		20		
Xgwm499	ACTTGTATGCTCCATTGATTGG	5BL	24	$(CA)_{34}$	54.65
	GGGGAGTGGAAACTGCATAA		22		
Xgwm508	GTTATAGTAGCATATAATGGCC	6BS	17	$(GA)_{32}$	49.75
	GTGCTGCCATGATATTT		23		
Xgwm550	CCCACAAGAACCTTTGAAGA	1B	20	$(GT)_{19}$imp	54.3
	CATTGTGTGTGCAAGGCAC		22		
Xwmc283	CGTTGGCTGGGTTATATCATCT	7A	22	$(CT)_8(GT)_{18}$	57.65
	GACCCGCGTGTAAGTGATAGGA		20		
Xwmc522	AAAAATCTCACGAGTCGGGC	2AS	20	(CA)19 64 to 101, (CA)8 104 to 119	56.4
	CCCGAGCAGGAGCTACAAAT		23		
Xwmc809	CAGGTCGTAGTTGGTACCCTGAA	7AL	19	(CT)36 74 to 145, (TC)4163 to 170	57.4
	TGAACACGGCTGGATGTGA		21		
Xcfa2099	TGCGAAGTATTCAGTGCGTC	2A	21	-	55.5
	TCAAGACCATCAGCACTCAGA		20		
Xcfa2174	ACGGCATCACAGGTTAAAGG	7A	20	$(CA)_{24}$ (C)	56.45
	GGTCTTTGCACTGCTAGCCT		20		
Xdupw38	ATTAGACACGACCAAACGGG	1A	20	$(CT)_{15}(GT)_{14}$	54.4
	TCAAACAAACAACAGCCAGC		21		
Xgpw2333	ACAAGCCCAAAAGACACACA	7A, 2B	20	(GCC)9	54.4
	ACATCACTTCCTCCGGTTTG		20		
Xuhw89	TCTCCAAGAGGGGAGAGACA	6B	23	$(CA)_{21}$	56.75
	TTCCTCTACCCATGAATCTAGCA			-	

Ch= chromosome, T (°C) = annealing temperature

Table S3. SSR markers associated with differents quality traits in previous studies.

Primers	Main phenotype	Reference
Xgwm471	Yellow pigment	Reimer et al. 2008
Xgwm371	Sedimentation test	Kerfalet al. 2010
	Thaousand kernel weight	Ramyaet al. 2010
Xgwm550	Sedimentation test	Patilet al. 2008b
Xwmc522	Dough strength.	Kerfalet al. 2010
	Yellow pigment	Reimer et al. 2008
	Protein content	Tang et al. 2013
Xgwm508	Protein content	Olmoset al. 2003
Xwmc283	Yellow pigment	Reimer et al. 2008
Xgwm299	Yellow pigment	Blanco et al. 2011
		Mares & Campbell 2001
		Howitt et al. 2009
		Patilet al. 2008a
		Reimer et al. 2008
	Protein content	Blanco et al. 2002
	Test weight	Jing et al. 2007
Xgwm46	Protein content	Patilet al.2008b
	Yellow pigment	Patilet al. 2008a
	Test weight	Patilet al. 2008b
Xgwm499	Thaousand kernel weight	Ramyaet al. 2010
		Kuchelet al.2006.
Xgwm344	Yellow pigment	Elouafi2003
Xgwm408	Yellow pigment	Patilet al.2008a
Xdupw38	Yellow pigment	Patilet al.2008a
	Test weight	Patilet al.2008b
Xgwm146	Yellow pigment	Zhang &Dubcovsky 2008
		Zhang et al.2008
	Protein content	Zhang et al.2008
Xcfa2174	Test weight	Patilet al.2008b
	Yellow pigment	Zhang &Dubcovsky 2008
	Protein content	Patilet al.2008b
Xuhw89	Protein content	Distelfeldet al.2006
Xgpw2333	Protein content	Patilet al.2008b
Xcfa2099	Protein content	Patilet al.2008b
Xwmc809	Yellow pigment	Singh et al.2009

Table S4. SSR marker allele size range

Primers	Allele N°	Size range (pb)	C.S. size (pb)
Xgwm471	2	142-152,1	152.1
Xgwm371	5	300-400	-
Xgwm550	4	241-243	-
Xwmc522	5	250-300	193
Xgwm508	2	198-200	198
Xwmc283	5	70-110	151
Xgwm299	3	210-228	208.3
Xgwm46	4	171-189	187
Xgwm499	4	110-225	124-184
Xgwm344	5	139-152	150
Xgwm408	3	149-183	148
Xdupw38	3	184-201	187
Xgwm146	4	148-184	-
Xcfa2174	7	250-300	191