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Pastamaking and breadmaking quality of soft-textured durum wheat lines

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ABSTRACT

The effects of grain texture on pastamaking and breadmaking quality were studied in three F_8 soft-textured durum wheat lines (SDLs) containing wild-type alleles *Pina-D1a* and *Pinb-D1a* as compared with their hard durum sister lines (HDLs). SDLs homozygous for a small 5DS segment, less than 14.4 cM in size, accumulated puroindolines A (Pin-A) and B (Pin-B) and showed SKCS values (19.9–23.6) significantly lower than those (72.6–76.8) of their hard-textured counterparts lacking Pin-A and Pin-B. In addition, SDLs exhibited approximately 24% higher flour extraction rates compared with HDLs. Reducing the kernel hardness decreased farinograph water absorption, dough tenacity (P) and, accordingly, alveograph *P*/*L* ratio, but increased farinograph stability, mixing tolerance and dough extensibility (*L*). Spaghetti cooking quality, as determined by the sensory judgment of firmness, stickiness and bulkiness, was unaffected by the kernel hardness, whereas the loaf volume exhibited a 10% increase associated with kernel softening. Flour and semolina, but not spaghetti, from SDLs showed a substantial reduction in yellowness (b^*) and brownness ($100 - L^*$) likely due to their finer particle size compared with HDLs. Alleles *Pina-D1a* and *Pinb-D1a* may offer new perspectives for breeding dual purpose (pasta and bread) durum wheat varieties.

1. Introduction

After common wheat (*Triticum aestivum* L.), durum wheat (*Triticum turgidum* ssp *durum*) is the second-most widely cultivated wheat species. Its extra-hard, translucent, light-coloured grain is mainly ground to make semolina for pasta and couscous. In Mediterranean countries, durum wheat is also used for breads of all types, including traditional flat breads and specialty breads (Quaglia, 1988). Compared with common wheat cultivars, currently grown durum wheat cultivars exhibit inferior breadmaking quality as measured in terms of loaf volume and crumb grain characteristics, and this has been attributed to the absence of the D-genome chromosomes (Kerber and Tipples, 1969). On the other hand, during the second half of the 20th century, breeding programs have focused on selecting durum wheat genotypes with superior pastamaking quality because of its primary commercial importance, and selection for baking quality has been applied to a minor extent (Boggini et al., 1995; Liu et al., 1996; Peña et al., 1994). However, there are durum wheat genotypes that make bread equal in volume and crumb texture to that of high quality common wheat (Boggini and Pogna, 1989; Edwards et al., 2007; Sapirstein et al., 2007). Durum wheats with good baking quality were found to possess low molecular weight glutenin subunits (LMW-GS) designated as LMW-2, along with high molecular weight glutenin subunits (HMW-GS) 7 + 8 or 6 + 8 (Boggini and Pogna, 1989; Edwards et al., 2007; Peña et al., 1994; Sapirstein et al., 2007; Sissons et al., 2005).

Bread wheat differs from durum wheat in kernel texture as well. In particular, common wheat cultivars can be divided into three endosperm-texture classes based on their average SKCS (Single Kernel Characterization System) values, i.e. soft (SKCS index = 15–40), medium hard (55–70) and hard (71–95). By contrast, all durum wheat cultivars are characterized by an extra-hard kernel texture with SKCS index >80, mainly due to the absence of two proteins named puroindolines A (Pin-A) and B (Pin-B). These are basic, tryptophan- and cysteine-rich polypeptides encoded by two closely linked genes named *Pina-D1* and *Pinb-D1*, which are located in the distal end of the short arm of chromosome 5D (Gautier et al., 1994; Mattern et al., 1973). Soft-textured common wheat cultivars possess wild-type alleles *Pina-D1a* and *Pinb-D1a* and accumulate large amounts of both Pin-A and Pin-B on the

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surface of their starch granules (Corona et al., 2001). On the contrary, hard- and medium hard-textured common wheat cultivars contain modified or null alleles at the *Pina-D1* and/or *Pinb-D1* loci and accumulate Pin-B on the starch granules in trace amounts, if any (Gautier et al., 1994). Therefore, Pin-A and Pin-B act as the principal determinant factors of endosperm texture in wheat and, as a consequence, exert a strong indirect impact on a bundle of quality traits including flour yield, granularity of flour, starch damage, water absorption, dough rheological properties, bread volume and crumb structure (Bhave and Morris, 2008). Pin-A and Pin-B are claimed to influence directly loaf volume and crumb structure as well (Igrejas et al., 2001).

To make a durum bread, semolina is reground to reduce its particle size and provide sufficient starch damage to assure appropriate gassing power during the fermentation process (Quaglia, 1988). Because of the extreme hardness of durum wheat grain, semolina regrinding can result in excessive starch damage, which alters alveogram and farinogram shapes, and exerts detrimental effects on baking performance (Dexter et al., 1994).

Gazza et al. (2003) used durum wheat line "Cappelli M" lacking the *Ph1* locus (Giorgi, 1978) as the female parent in a cross with the 5D(5B) substitution line of durum wheat cv. Langdon carrying wild-type alleles *Pina-D1a* and *Pinb-D1a*. Due to the absence of the *Ph1* locus in the progeny from this cross, pairing and allosyndetic recombination between homoeologous group 5 chromosomes resulted in soft-textured plants devoid of chromosome 5D. These plants were used as the male parent in crosses with commercial durum wheat cv. Colosseo (Gazza et al., 2008), and three F_6 progeny hemizygous for *Pina-D1a* and *Pinb-D1a* were self-pollinated for two generations to develop six F_8 lines, i.e. three Soft Durum Lines (SDL1, SDL2 and SDL3) homozygous for *Pina-D1a* and *Pinb-D1a*, and three Hard Durum Lines (HDL1, HDL2 and HDL3) lacking the *Pina-D1* and *Pinb-D1* loci.

In the present work, SDL1, SDL2, SDL3 HDL1, HDL2 and HDL3 grown in replicated plots in 2009 and 2010 at Montelibretti, Rome, Italy, are compared for their milling properties, rheological characteristics, pastamaking and breadmaking quality.

2. Materials and methods

2.1. DNA extraction and PCR amplification

DNA was extracted from leaves by the CTAB method. Puroindoline genes were amplified by PCR as described by Gautier et al. (1994). SSR (Simple Sequence Repeat) sequences on chromosome 5D were used for microsatellite marker characterization (Somers et al., 2004; Song et al., 2005).

2.2. Electrophoretic analyses

Starch-bound proteins were extracted with 50 mM NaCl and 50% (v/v) propan-2-ol from 50 mg of air-dried starch granules and fractionated by A-PAGE at pH 3.1 as described by Corona et al. (2001). Reduced endosperm proteins were fractionated by SDS-PAGE as described previously (Pogna et al., 1990).

2.3. Agronomic trials

Three soft-textured durum lines named SDL1, SDL2 and SDL3, and their hard-textured counterparts HDL1, HDL2 and HDL3 were grown in 2009 and 2010 in the Tiber Valley at Montelibretti (Rome) on sandy soil. The experimental design was a randomized block with three replications. The elementary plot of 10 m^2 consisted of eight rows, 17 cm apart, sown with 400 germinating kernels/m^2. The husbandry conditions were those used for commercial production, including 150 kg/ha of N applied in three top-dressings. Plant height, heading time, grain yield and hectoliter weight were measured on each plot. Seeds from the three replications of each line were blended and assessed for their quality characteristics according to the methods given in Table 1.

2.4. Kernel hardness and milling

Kernel hardness was performed on 300 kernels-sample by the Perten SKCS 4100 (Springfield, IL, USA) following the manufacturer's operating procedure. The instrument was set in a range of hardness values between −40 and +120. Samples (3 kg) from soft-textured and hard-textured lines were tempered overnight to 16.0% or 16.5% moisture respectively, and milled with (i) the MCK Buhler experimental mill for durum wheat (procedure 7, Table 1), (ii) the MLU 202 Buhler experimental mill for common wheat (procedure 8) or (iii) the Bona 4RB (Bona, Monza, Italy) experimental mill for common wheat (procedure 9). To determine ash content, samples were incinerated overnight in a muffle furnace at 600 °C according to the AACC Method 08-01.01.

2.5. Rheological tests

The milled samples obtained with procedures 7 and 8 were analysed with the Chopin Alveograph (Chopin, Villeneuve La Garenne, France) according to the manufacturer's instructions as modified by D'Egidio et al. (1990), and with the Brabender (South Hackensack, NJ, USA) farinograph according to the AACC method 54.21-01.

2.6. Pastamaking

Flour (particle size ≤ 282 μm) and semolina (particle size > 282 μm) obtained from each soft-textured or hard-textured line with the MCK Buhler experimental mill for durum wheat (procedure 7) were combined to give a product referred to hereafter as "granulars", and mixed with tap water to reach a dough water content of 24.5% (for SDLs) or 30% (for HDLs). The dough was processed into spaghetti (1.7 mm in diameter) using a laboratory press (Serma, Milan, Italy) with a capacity of 1.5–3.0 kg. After drying at 50 °C for 20 h, spaghetti (100 g) were cooked and evaluated for firmness (resistance to chewing by the teeth), stickiness (amount of material adhering to the spaghetti surface) and bulkiness (adhesion of pasta strands to each other) by a trained panel of

Table 1
Procedures used for quality measurements.[a]

No.	Procedure	Measurements
1	Infratec 1241 Grain Analyser	Hectoliter weight
2	From a 20 g kernel sample	1000 kernel weight
3	AACC Method 55-31.01	SKCS
4	Infratec 1241 Grain Analyser	Seed protein
5	AACC Method 56-70.01	SDS sedimentation volume
6	AACC Method 56-81.03	Falling number
7	D'Egidio et al. (1990)	Milling yield
8	AACC Method 26-21.02	Milling yield
9	Bona Method	Milling yield
10	AACC Method 08-01.01.	Ash content
11	D'Egidio et al. (1990)	Alveograph (*W*, *P* and *L*)
12	AACC Method 54-21.01	Farinograph (water absorption, DT, stability, MTI)
13	Chroma CR-300 m	b^* and L^*
14	AACC Method 14-50.01	Carotenoid content
15	AACC Method 10-10B	Bread volume, height and weight
16	D'Egidio et al. (1990)	Sensory judgment of spaghetti

[a] For the AACC methods see www.aaccnet.org/ApprovedMethod/.

three experts as described by D'Egidio et al. (1990). The Sensory Judgment (SJ) was calculated as the arithmetic mean of the scores for firmness (10–20 = absent, 21–40 = rare, 41–60 = sufficient, 61–80 = good, and 81–100 = very good), stickiness and bulkiness (10–20 = very high, 21–40 = high, 41–60 = rare, 61–80 = minimal, and 81–100 = absent).

2.7. Breadmaking

Bread was baked according to the AACC Method 10-10B except that no milk or potassium bromate was added, using flour samples obtained with the milling procedure 8 for common wheat. The water absorption determined by the farinograph was used to reach the optimum bake absorption for each sample. Loaf volume was determined by rapeseed displacement.

2.8. Colour analysis and β-carotene content

Colour was evaluated by measuring brightness (L^*) and yellow index (b^*) with the CR-300 Chroma meter (Minolta, Osaka, Japan) on flour, semolina and granulars obtained with the milling procedure 7 for durum wheat, as well as on dry, uncooked spaghetti mounted as a 5-cm-wide band on a white cardboard using double-sided tape or ground in a Cyclone Mill (Tecator AB, Sweden), fitted with a 0.5 mm sieve (D'Egidio et al., 1990). Granulars were analysed for their pigment contents by the AACC Method 14-50.01, using the wavelength for β-carotene.

2.9. Statistics

Data are the means of at least duplicate determinations, with the exception of milling, falling number, farinograph, alveograph and cooking test, which were determined singly. Data were statistically evaluated by Student's t test or analysis of variance.

3. Results

3.1. Protein composition and genetic characterization of durum wheat lines

Upon A-PAGE fractionation (Fig. 1), soft-textured durum wheat lines SDL1, SDL2 and SDL3 were found to accumulate Pin-A and Pin-B on the surface of their starch granules in amounts similar to those observed in soft-textured common wheat cv. Bolero (lane 4) and Langdon 5D(5B) substitution line (lane 3). By contrast, hard-textured durum wheat lines HDL1, HDL2 and HDL3 were similar to durum wheat varieties in lacking both puroindolines (lanes 5 and 6). According to SDS-PAGE, all the soft-textured and hard-textured durum wheat lines in the present study exhibited LMW-2 glutenin subunits, which are associated with superior gluten strength (Pogna et al., 1990). Moreover, they inherited HMW glutenin subunit pair 6 + 8 from Langdon 5D(5B) substitution line (data not shown).

PCR amplifications of genomic DNA from each durum line with primer pairs specific for microsatellites *Xbarc130* (chromosome 5DS, 0.0 cM), *Xbarc190* (5DS, 8.6 cM), *cfd189* (5DS, 14.4 cM), *Xbarc205* (5DS, 18.5 cM), *Xbarc44* (5DL, 34.4 cM), *Xbarc320* (5DL, 94.2 cM) and *Xbarc 110* (5DL, 140.4 cM) produced amplicons of the expected sizes in Langdon 5D(5B) substitution line, but failed to give an amplification fragment in HDL1, HDL2, HDL3 and their durum wheat parental cvs Cappelli M and Colosseo. On the other hand, all the soft-textured durum wheat lines gave strong amplicons of the expected sizes exclusively with primers specific for microsatellites *Xbarc130* and *Xbarc190*. The former SSR is closely linked to the *Ha* locus harbouring the *Pina-D1* and *Pinb-D1* genes on

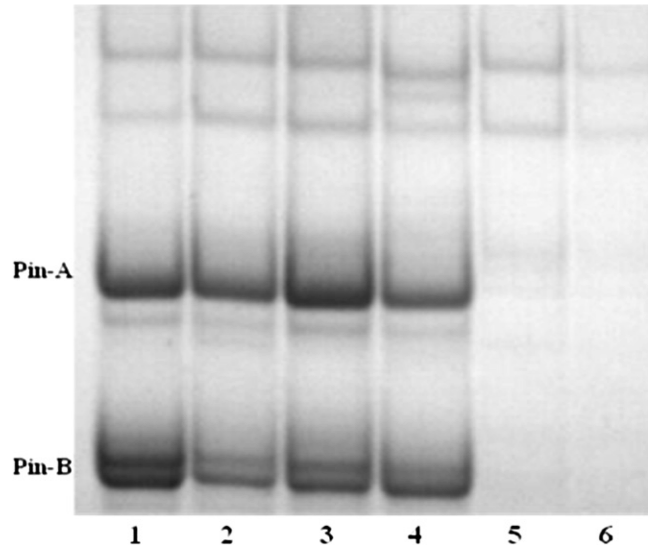

Fig. 1. A-PAGE fractionation of puroindolines (Pin-A and Pin-B) in (1) Soft Durum Line (SDL) 1; (2) SDL2; (3) Langdon 5D(5B) substitution line; (4) bread wheat cv. Bolero; (5) Hard Durum Line (HDL)1 and (6) HDL 2.

the distal end of chromosome 5DS, whereas *Xbarc190* has been mapped at 8.6 cM from the *Ha* locus (Song et al., 2005).

SDL1 was crossed with durum wheat cv. Simeto and the segregation of allele *Pina-D1a* coding for wild-type Pin-A was followed by PCR amplification of DNAs extracted from single F_2 plants obtained from this cross. Segregation of kernel texture as determined by SKCS analysis of 100 F_3 kernels produced by each F_2 plant was followed as well. The combined results for the 132 progeny of this cross showed that the two phenotypic classes for allele *Pina-D1a* (105 positive plants *vs* 27 negative plants) and the three phenotypic classes for kernel texture (32 soft progeny with SKCS <30; 73 intermediate progeny with SKCS = 49 to 72; 27 hard progeny with SKCS ≥ 82) occurred in frequencies very close to the 3:1 and 1:2:1 ratios, respectively. In addition, the 27 progeny lacking *Pina-D1a* exhibited mean SKCS values ≥ 82 and Standard Deviations (SD) ≤ 17, which were comparable with those of cv. Simeto (SKCS = 79 ± 14). Relatively low SDs (≤ 16) were also observed in the 32 F_3 progeny with soft kernels (mean SKCS $\leq$ 30), whereas the 73 progeny with intermediate kernel texture had SDs as high as 29 to 37. These observations suggest that *Pina-D1a* in the parental SDL1 was transmitted to the F_2 and F_3 generations without any major distortion in normal segregation, and that the presence of allele *Pina-D1a* resulted in F_3 kernels having reduced SKCS values.

3.2. Agronomic performance

No significant differences in yield, plant height, heading time and hectoliter weight were observed between SDLs and HDLs in the two agronomic trials (Table 2). Plant height in SDLs, HDLs and parental cv. Cappelli M was high (>120 cm) and resulted in partial (30–60%) lodging at harvesting in both years of testing. Moreover, kernels harvested in 2010 revealed mean hectoliter weights lower than 75.0 kg hL^{-1}. Compared with the parental cv. Colosseo cultivated in adjacent experiments, SDLs and HDLs showed a yield penalty of approximately 9% on average (data not shown).

3.3. Kernel and flour characteristics

The soft-textured durum lines could not be differentiated statistically from the hard-textured lines for kernel weight and

Table 2
Mean ± SD and *t*-value for agronomical and quality measurements carried out during two years of testing on six durum wheat lines with contrasting grain textures.

Measurements	SDL[a]			HDL[b]			*t*-value[c]
	2009	2010	Grand mean	2009	2010	Grand mean	
Yield (t/ha)	5.7 ± 0.6	4.6 ± 0.8	5.1 ± 0.9	5.8 ± 0.5	4.4 ± 0.7	5.1 ± 0.9	0.3 ns
Plant height (cm)	120 ± 2.0	122 ± 2.1	121 ± 2.1	122 ± 1.6	124 ± 1.8	123 ± 2.1	0.3 ns
Heading time (days)[d]	28 ± 3.6	27 ± 3.1	27.5 ± 3.2	29 ± 3.1	30 ± 3.4	29.5 ± 3.0	1.6 ns
Hectoliter wt (kg hL^{-1})	80.0 ± 0.1	73.9 ± 0.3	76.9 ± 3.3	80.1 ± 0.2	74.8 ± 02	77.4 ± 2.9	0.5 ns
1000-kernel wt (g)	44.8 ± 1.8	40.5 ± 0.9	42.6 ± 2.2	44.1 ± 1.5	41.7 ± 2.4	42.9 ± 2.2	0.2 ns
SKCS index	19.9 ± 6.0	23.6 ± 3.2	21.8 ± 4.7	76.8 ± 1.2	72.6 ± 2.3	74.7 ± 2.8	23.4**
Seed protein (%, dry wt)	15.6 ± 0.1	16.2 ± 0.1	15.9 ± 0.3	15.4 ± 0.2	15.9 ± 0.5	15.6 ± 0.4	1.2 ns
Sedimentation (ml)	79.0 ± 1.5	84.0 ± 1.7	81.0 ± 3.1	64.0 ± 2.0	63.0 ± 2.0	64.0 ± 1.9	12.6**
Falling number (s)	515 ± 39.5	467 ± 30.5	491 ± 41.1	497 ± 45.9	553 ± 50.0	525 ± 52.7	1.4 ns
Milling[e]							
Flour yield (%)	26.6 ± 0.1	29.7 ± 0.2	28.1 ± 1.7	16.6 ± 0.7	18.4 ± 3.0	17.5 ± 2.2	9.3**
Semolina yield (%)	32.8 ± 1.5	35.5 ± 1.9	34.2 ± 2.1	44.3 ± 4.2	48.4 ± 1.4	46.3 ± 3.6	7.2**
Granulars yield (%)	59.4 ± 1.6	65.2 ± 2.1	62.3 ± 3.2	60.9 ± 4.8	66.8 ± 4.5	63.8 ± 5.3	0.6 ns
Granulars ash (%, dry wt)		0.9 ± 0.3			1.1 ± 0.1		1.1 ns
Milling[f]							
Flour yield (%)	68.1 ± 1.4	68.0 ± 0.3	68.0 ± 0.9	54.4 ± 0.6	55.2 ± 0.1	54.8 ± 0.6	30.5**
Farinograph							
Develop. time (min)	1.5 ± 0.2	1.6 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	0.0 ns
Stability (min)	6.8 ± 0.6	3.7 ± 0.6	5.2 ± 1.8	4.4 ± 0.1	1.8 ± 0.1	3.1 ± 1.4	2.3*
MTI (BU)	42 ± 2.0	68 ± 5.0	55 ± 14.3	68 ± 2.5	89 ± 1.5	78 ± 11.6	3.1*
Water absorp. (%)	53.6 ± 0.1	58.0 ± 0.6	55.8 ± 2.4	66.7 ± 0.2	70.5 ± 0.4	68.6 ± 2.1	10.6**
Alveograph							
W (J × 10^{-4})	221 ± 19.1	172 ± 33.2	196 ± 36.1	291 ± 12.4	261 ± 30.0	276 ± 26.3	4.3**
P (mm)	64 ± 0.6	83 ± 1.1	73 ± 10.4	97 ± 13.3	121 ± 10.3	109 ± 16.9	4.3**
L (mm)	104 ± 9.3	66 ± 2.1	85 ± 22.2	90 ± 12.7	61 ± 4.6	76 ± 18.0	0.8 ns
P/L	0.6 ± 0.1	1.2 ± 0.1	0.9 ± 0.3	1.1 ± 0.3	2.0 ± 0.3	1.4 ± 0.6	2.3*
Spaghetti cooking test							
Firmness index	74.0 ± 1.0	73.0 ± 3.6	73.5 ± 2.4	73.0 ± 1.0	75.7 ± 6.0	74.3 ± 4.1	0.4 ns
Stickiness index	31.7 ± 1.5	44.7 ± 12.7	38.2 ± 10.8	33.0 ± 1.1	61.0 ± 12.2	47.0 ± 17.2	1.1 ns
Bulkiness index	50.0 ± 2.0	57.0 ± 1.7	53.5 ± 4.2	50.0 ± 0.9	62.3 ± 11.0	56.2 ± 9.7	0.6 ns
Sensory judgment	51.9 ± 0.8	58.2 ± 5.7	55.1 ± 5.1	52.0 ± 1.2	66.3 ± 9.5	59.2 ± 9.9	0.9 ns

*,**Significant at $P = 0.05$ and $P = 0.01$, respectively; ns = not significant.
[a] Soft Durum Lines 1, 2 and 3.
[b] Hard Durum Lines 1, 2 and 3.
[c] 34 d*f* for yield, plant height, heading time and hectoliter weight; 10 d*f* for the remaining measurements, except ash content (4 d*f*).
[d] Number of days from April1st.
[e] Procedure 7 (Table 1).
[f] Procedure 9 (Table 1).

falling number (Table 2). This latter trait was >450 s on average, indicating that the samples were not sprouted. By contrast, in the two years of testing, the hardness indexes of soft-textured SDLs varied from 19.9 to 23.6, significantly lower than those reached by HDLs (72.6–76.8) and their parental durum cvs Colosseo (82.5–88.3) and Cappelli M (76.8–77.3). Interestingly, the soft-textured lines showed an average increase of approximately 1.25-fold in the SDS sedimentation volume when compared with their HDL counterparts. This result was not explained by the minuscule difference in protein content between SDLs (grand mean = 15.9 ± 0.3%) and HDLs (15.6 ± 0.4%).

Because of the unusual soft characteristics of SDL grain, samples were milled with experimental mills for durum wheat (MKC Bhuler mill) and common wheat (Bona mill). Extraction rates of flour (particle size ≤ 282 μm) and semolina (particle size > 282 μm) obtained with the MCK Buhler mill showed considerable variation as would be expected from wheat genotypes with contrasting kernel texture characteristics (Table 2). In particular, SDLs had about 60% higher flour yield and approximately 26% lower semolina yield compared with HDLs across all trials. However, extraction rate of granulars (flour and semolina combined) averaged 63.1%, ranging from 62.3 ± 3.2% for SDLs to 63.8 ± 5.3% for HDLs, this variation being non-significant statistically. The ash content of granulars milled from wheats grown in 2010 was approximately 22% higher in HDLs when compared with SDLs. In addition, the ash contents in SDL1 (0.7%) and SDL2 (0.8%) were remarkably lower than that of SDL3 (1.3%), which explains the relatively high coefficient of variation (33%) in the Soft Durum Lines, and the non-significant *t* value for this milling trait. Finally, milling with the Bona 4RB mill for common wheat (procedure 9) resulted in flour yields ranging from 54.4% to 68.1%, with a clear evidence of genotypic effects. In fact, SDLs exhibited about 24% higher extraction rates when compared with HDLs.

3.4. Rheological tests

As expected, the hard kernel texture of HDLs had significant impact on farinograph water absorption of granulars obtained with the MCK Buhler mill (Table 2). Averaged over all trials, uptake of water by HDL granulars (68.6 ± 2.1%) was about 23% higher than that of SDL granulars (55.8 ± 2.4%), likely reflecting the high starch damage of the milling products from hard kernels compared with soft kernels. Moreover, farinograph stability of HDL granulars (grand mean value of 3.1 ± 1.4 min) showed a significant decrease with respect to SDL granulars (5.2 ± 1.8 min), which was likely due to the inability of damaged starch in HDL granulars to hold all the water absorbed initially. On average, mixing tolerance as shown by mixing tolerance index (MTI) was significantly low in HDLs (MTI = 78 ± 11.6 BU) compared with SDLs (MTI = 55 ± 14.3 BU). Finally, dough development time was rather short (1.5–1.6 min) in both groups of durum lines.

The soft-textured genotypes could be easily differentiated from the hard-textured lines for all the alveograph properties of their granulars (Table 2). Alveograph P and W values, which are indicators of dough elasticity and “strength” (the energy required to blow and break a bubble of dough), respectively, were significantly higher in HDLs compared with SDLs in both years of testing. On the contrary, there was a trend for HDLs to have consistently, but not significantly, low alveograph *L* values (an indicator of dough extensibility), which resulted in high *P*/*L* ratios (grand mean $P/L = 1.4 \pm 0.6$) compared with SDLs (0.9 ± 0.3). This ratio is a measurement of the balance between elasticity and extensibility and, with some exceptions, is higher than 1.0 in durum wheat (Quaglia, 1988), well reflecting the tenacious inextensible dough properties of this wheat species.

3.5. Pastamaking quality

Appearance and cooking quality of pasta produced with granulars obtained with the MCK Buhler mill were not significantly affected by the contrasting grain textures of SDLs and HDLs (Table 2). However, stickiness was slightly higher in SDLs (grand mean stickiness index $= 38.2 \pm 10.8$) compared with HDLs (47.0 ± 17.2), this difference being statistically non-significant due to the pronounced effect exerted by the second year (2010) of testing on this character. The global quality scores of pasta for HDLs (grand mean SJ $= 59.2 \pm 9.9$) and SDLs (55.1 ± 5.1) did not differ significantly, and were comparable with those observed in good-quality durum wheat cultivars grown in Italy.

3.6. Colour analysis

Flour, semolina and granulars obtained from SDLs and HDLs milled with the MCK Buhler mill were scored for colour using a Minolta Chroma CR-200 m and colour readings were expressed as b^* (a measure of the yellowness) and L^* (lightness), using $(100 - L^*)$ as a measure of the brownness.

The milling fractions (flour, semolina and granulars) of the hard-textured lines exhibited significantly higher yellow and brown indexes compared with their soft-textured counterparts (Table 3). The pigment content of granulars was determined by the AACC Method 14-50.01 immediately after grinding without any flour storage in order to reduce the bleaching activity of lipoxygenase, and expressed as ppm of β-carotene. Surprisingly, when compared with HDLs, granulars from SDLs revealed an average increase of approximately 1.7-fold in the amount of carotenoids. The classic ANOVA (Table 4) showed a significant effect of kernel texture and milling fraction on both colour indexes. Year of testing and two-way interactions between kernel texture, milling fraction and year affected slightly, but significantly, brownness and/or yellowness as well. Uncooked spaghetti mounted on a white cardboard exhibited high yellow indexes, with non-significant variation between hard-textured (grand mean $= 23.8 \pm 0.5$) and soft-textured (22.8 ± 0.9) genotypes (Table 3). Grinding of uncooked spaghetti by the Cyclone mill had an impact on yellowness and resulted in a significantly high yellow index in HDLs (grand mean $b^* = 16.7 \pm 0.7$) compared with SDLs (14.4 ± 0.4). Finally, hard-textured and soft-textured lines did not differ significantly in the brownness of their spaghetti or ground spaghetti.

3.7. Breadmaking quality

Kernels of HDL1, HDL2, SDL1 and SDL2 harvested in 2010 were milled with the MLU 202 Buhler mill for common wheat (procedure 9) and the resulting flour samples were characterized for their rheological properties and compared in a baking test according to the AACC Method 10-10B with minor modifications. The two soft-textured lines had about 23% higher flour extraction rates and 18% lower farinograph water absorptions compared with the hard-textured genotypes (Table 5). As observed in granulars obtained with the MCK Buhler mill for durum wheat (Table 2), farinograph stability and mixing tolerance (as measured by MTI) were significantly higher for soft lines than hard lines, whereas development time was identical (Table 5). Tenacity (*P*) and length (*L*) were the alveograph measurements that were highly affected by kernel texture, confirming that SDLs are characterized by higher extensibility (*L*) and reduced tenacity (*P*) compared with HDLs. As a consequence, alveograph *P*/*L* of these latter lines (mean $P/L = 8.0$) was extremely high compared with that of SDLs ($P/L = 1.7$), which corresponds to an increase of roughly 4.7-fold. Due to the opposite effects of puroindolines on *P* and *L*, the energy (*W*) required to blow the alveograph bubbles of SDLs was similar to that of HDLs.

Variation in kernel texture resulted in minor effects on baking performance. SDLs exhibited slightly, but significantly, higher bread loaf volumes, but did not differ from HDLs in bread weight and height (Table 5).

4. Discussion

In most rainfed areas of the Mediterranean region under stressful environmental conditions, durum wheat is the preferred cereal crop due to its superior adaptation to high temperatures

Table 3
Mean value (±SD) for brown index $(100 - L^*)$, yellow index (b^*) and carotenoid content in six durum wheat lines with contrasting grain textures during two years of testing.

Measurements	SDL[a]			HDL[b]			*t*-value[c]
	2009	2010	Grand mean	2009	2010	Grand mean	
Brown index $(100 - L^*)$							
Flour	7.1 ± 0.1	8.1 ± 0.5	7.6 ± 0.6	12.1 ± 0.2	13.2 ± 0.2	12.6 ± 0.6	13.8**
Semolina	9.8 ± 0.1	12.3 ± 0.8	11.0 ± 1.4	13.7 ± 0.4	14.0 ± 0.6	13.9 ± 0.5	4.5**
Granulars[d]	8.5 ± 0.1	9.7 ± 0.5	9.1 ± 0.8	12.6 ± 0.2	13.7 ± 0.1	13.2 ± 0.6	10.3**
Spaghetti	45.5 ± 0.8	48.7 ± 2.4	47.1 ± 2.4	47.3 ± 0.7	48.0 ± 0.7	47.6 ± 0.7	0.5 ns
Ground spaghetti	16.1 ± 0.6	17.7 ± 2.1	16.9 ± 1.6	17.0 ± 0.2	19.1 ± 0.4	18.1 ± 1.2	1.4 ns
Yellow index b^*							
Flour	7.8 ± 0.7	8.8 ± 0.1	8.3 ± 0.5	13.2 ± 0.6	13.1 ± 0.7	13.2 ± 0.6	12.9**
Semolina	11.2 ± 0.6	13.3 ± 0.1	12.3 ± 1.3	17.6 ± 0.6	18.6 ± 0.7	18.1 ± 0.8	9.9**
Granulars[d]	9.1 ± 0.4	10.1 ± 0.6	9.6 ± 0.7	15.8 ± 0.6	16.5 ± 0.3	16.1 ± 0.6	17.6**
Spaghetti	22.7 ± 0.9	23.0 ± 1.0	22.8 ± 0.9	24.0 ± 0.4	23.7 ± 0.7	23.8 ± 0.5	1.7 ns
Ground spaghetti	14.1 ± 0.1	14.7 ± 0.4	14.4 ± 0.4	16.1 ± 0.1	17.3 ± 0.1	16.7 ± 0.7	7.0**
β-carotene (ppm)							
Granulars[d]	2.3 ± 0.1	2.7 ± 0.2	2.5 ± 0.3	1.6 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	7.6**

**Significant at $P = 0.01$, respectively; ns = not significant.
[a] Soft Durum Lines 1, 2 and 3.
[b] Hard Durum Lines 1, 2 and 3.
[c] $df = 10$.
[d] Flour and semolina combined as obtained by the milling procedure 7 (Table 1).

Table 4
Mean squares for yellow index (b^*) and brown index ($100 - L^*$) in the milling fractions from six durum wheat lines grown during two years of testing.

Source of variation	Mean squares	
	b^*	L^*
Grain texture[a] (*T*)	297.6**	156.7**
Milling fraction[b] (*F*)	120.6**	28.3**
Year (*Y*)	7.9**	13.6**
$T \times F$	4.3**	5.2**
$T \times Y$	1.6**	2.7**
$F \times Y$	2.1**	0.7 ns
$T \times F \times Y$	0.3 ns	4.2**
Error	0.2	6.4

**Significant at $P = 0.01$; ns = not significant.
[a] Soft *vs* hard.
[b] Flour and semolina.

(≥30 °C) and sub-optimal moisture conditions. Changes in global climate are forecast to increase the extension of hot, drought-prone areas, which could promote the diffusion of durum wheat cultivation. This wheat species is the raw material of choice for pasta and couscous (Quaglia, 1988), and is also used for flat breads and specialty breads. Unfortunately, suitability of most durum wheat varieties for making high-volume hearth bread remains inferior to that of common wheat because of their tenacious inextensible dough properties, which negatively affect oven response and loaf volume.

The absence of chromosome 5DS in durum wheat greatly increases its kernel hardness (Pogna et al., 2002) and is considered partly responsible for the relatively poor baking quality of durum wheat. The soft-textured durum F_8 lines SDL1, SDL2 and SDL3 analysed here were found to be of particular interest in elucidating the relationship between kernel texture and end-use quality in durum wheat.

PCR amplifications with primer pairs specific for seven SSRs on chromosome 5D suggested that SDLs contain a small 5DS fragment, less than 14.4 cM in size, likely translocated to homoeologous chromosome 5BS (data not shown). The wild-type *Pina-D1a* allele (and, very likely, the closely linked *Pinb-D1a* allele) on this DNA fragment was found to be regularly inherited in the 1:2:1 ratio in the 132 F_2 progeny of the cross between SDL1 and durum wheat cv. Simeto, and to modulate their kernel texture accordingly. The present findings also suggest that Pin-A and Pin-B accumulated on the surface of starch granules of SDL1, SDL2 and SDL 3 in amounts comparable to those of soft-textured wheat cv. Bolero (Fig. 1), and reduced their mean SKCS indexes to 19.9–23.6, which are typical of soft-textured common wheat cultivars (Table 2).

Table 5
Mean value (±SD) for rheological measurements and bread characteristics of four durum wheat lines with contrasting grain textures.

Measurements	SDL[a]	HDL[b]	*t*-value[c]
Milling[d]			
Flour yield (%)	67.9 ± 0.5	55.2 ± 0.4	28.0**
Farinograph			
Development time (min)	1.6 ± 0.2	1.6 ± 0.2	0.0 ns
Stability (min)	3.6 ± 0.4	1.8 ± 0.4	4.5*
MTI (BU)	68.0 ± 5.1	87.0 ± 1.4	5.1*
Water absorption (%)	57.5 ± 0.7	70.5 ± 0.6	19.9**
Alveograph			
W ($J \times 10^{-4}$)	262 ± 24.0	258 ± 23.5	0.2 ns
P (mm)	109 ± 1.4	215 ± 1.3	78.5**
L (mm)	68.5 ± 7.8	27.0 ± 4.3	6.6*
P/*L*	1.7 ± 0.2	8.0 ± 0.3	24.7**
Bread			
Volume (ml)	589 ± 16.0	533 ± 7.3	4.5*
Height (cm)	8.9 ± 0.3	8.4 ± 0.4	1.4 ns
Weight (g)	146 ± 3.7	153 ± 4.1	1.8 ns

*,**Significant at $P = 0.05$ and $P = 0.01$, respectively; ns, not significant.
[a] Soft Durum Lines 1 and 2.
[b] Hard Durum Lines 1 and 2.
[c] d*f* = 2.
[d] Procedure 8 (Table 1).

When compared with their hard-textured counterparts, SDLs revealed that grain hardness has a strong influence on several quality-related traits at the tetraploid level as well. In particular, the average flour extraction rate of SDLs milled with the Bona 4RB or MLU 202 Buhler mill for bread wheat was approximately 23–24% higher than that of HDLs, and even greater (about 60%) after milling with the MCK Buhler mill for durum wheat. These results agree with those of Campbell et al. (2001) and Tsilo et al. (2011), indicating the significant effect of the hardness *Ha* locus on flour yield in common wheat. The latter authors also reported a QTL at <15.6 cM from *Ha*, which accounted for 8.3% of the phenotypic variation in flour ash content. Two out of three SDLs in the present study were found to be approximately 32% lower in granulars ash content with respect to HDLs, suggesting a possible association between the 5DS fragment in the soft-textured durum lines and a reduced ash content.

Grain softness strongly decreased farinograph water absorption as well. When the granulars produced with the MCK Buhler mill were submitted to the alveograph test at constant hydration, irrespective of the damaged starch content of the milling products, variation in water absorption resulted in inferior dough tenacity (*P*), strength (*W*) and *P*/*L* ratio of SDLs with respect to HDLs (Table 2). The influence of water absorption on dough tenacity and *P*/*L* ratio was confirmed by the alveograph test performed on grain milled with the MLU 202 Buhler mill for common wheat (Table 5), and was in agreement with the results obtained in bread wheat (Bordes et al., 2008; Branlard et al., 2001). In this rheological test on flour, variation in kernel hardness had high influence on dough extensibily (*L*) as well, likely because of the high starch damage of flour compared with granulars. Moreover, the lower starch damage accounts for the higher farinograph dough stability and mixing tolerance of SDL milling products (granulars and flours), which is a likely outcome of their lower water absorption (Tables 2 and 5).

The yellowness of durum wheat semolina is an important trait that affects the colour of end-use products and the consumer's choice (Feillet et al., 2000). This trait is largely determined by the accumulation of carotenoids in the endosperm, principally the hydroxylated carotenoid lutein, and α- and β-carotene. In addition, lipoxygenase activity is responsible for the oxidative degradation of carotenoid pigments during pasta processing, whereas peroxidase and polyphenoloxidase activities contribute the brown colour of semolina, reducing its lightness (Borrelli et al., 2003). As compared with HDLs, the soft texture of SDLs resulted in significantly lower yellow and brown indexes in both flour and semolina fractions obtained with the MCK Buhler mill (Table 3). ANOVA showed that b^* and L^* varied significantly due to grain texture and milling fraction, with relatively small effects due to environment (year) and texture-by-fraction interaction. This is a clear indication that colour was strongly related to the particle size of the milling fractions, yellowness b^* and brownness ($100 - L^*$) being consistently and significantly lower for the finer flours and semolinas of SDLs. Moreover, the higher ash amount in HDL granulars could lead to an increased brown hue (Feillet et al., 2000). On the other hand, spaghetti from all of the durum wheat lines in this study exhibited a bright yellow colour, independently of their contrasting texture characteristics (Table 3). Interestingly, the grinding treatment of spaghetti resulted in a significantly higher b^* index in HDLs with respect to SDLs, confirming the role played by the particle size in determining this colour parameter. Recently, a QTL coinciding with the *Ha* locus has been found to explain a substantial proportion of the total phenotypic variation in b^* (13.7%) and L^*(22%) observed in a common wheat mapping population of 139 recombinant inbred lines (Tsilo et al., 2011).

Finally, the significant higher carotenoid content of granulars from SDLs compared with those from HDLs, as measured in terms of β-carotene concentration (Table 3), could be accounted for by a higher extraction efficiency attributable to the greater surface area of the finer particles in SDL granulars. Similarly, the fine particles in the milling products of SDLs could explain the high SDS sedimentation volumes of these soft-textured genotypes when compared with HDLs. However, these findings deserve further investigation.

It is noteworthy that the substantial variation in milling yield, water absorption and rheological properties associated with the contrasting kernel textures of the durum wheat lines in this study did not significantly affect firmness, stickiness and bulkiness of their spaghetti (Table 2), and resulted in a small, but significant, increase of the bread loaf volume (approximately by an average of 10%) in soft-textured lines compared with their hard-textured counterparts (Table 5). These results suggest that modulation of kernel hardness in durum wheat does not impair its pastamaking potential and may even improve its baking performance.

There is a close relationship between grain hardness and energy consumption during milling (Pomeranz and Williams, 1990). Moreover, grain hardness affects a plethora of factors of industrial interest such as flour yield, starch damage level, colour and water absorption of flour/semolina, end product performance and quality. For all these reasons, availability of soft-textured durum wheat genotypes may have important practical and useful implications for breeding dual purpose (pasta/bread) varieties and for technological durum wheat operations.

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