Rendiconti
Accademia Nazionale delle Scienze detta dei XL
Memorie di Scienze Fisiche e Naturali
136° (2018), Vol. XLII, Parte II, Tomo I, pp. 95-101

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Breeding and quality of soft-textured durum wheat

Summary – Kernel hardness in common wheat exerts a strong impact on yield and granularity of flour, starch damage and water absorption, rheological and baking properties. Durum wheat cultivars are characterized by an extra-hard kernel. This extremely hard texture is mainly due to the absence of puroindolines A and B, two basic, tryptophan- and cysteine-rich polypeptides encoded by two closely linked genes named *Pina-D1* and *Pinb-D1*, located in the short arm of chromosome 5D. In order to insert puroindoline genes, durum wheat line «Cappelli M» lacking the *Ph1* locus was used as the female parent in a cross with the 5D(5B) substitution line of durum wheat cv Langdon carrying puroindolines wild-type alleles. F9 lines, three Soft Durum Lines (SDL) homozygous for wild-type alleles *Pina-D1a* and *Pinb-D1a*, and three Hard Durum Lines (HDL), lacking the *Pina-D1* and *Pinb-D1* genes, were selected. Microsatellites located on 5D chromosome suggested that SDLs contain only a small 5DS fragment, inferior to 14.4 cM in size. Accumulation of puroindolines reduced SDLs hardness indexes to those typical of soft-textured common wheat cultivars. Flour extraction rate of SDLs was approximately 60% higher than that of HDLs and grain softness strongly decreased farinograph and alveograph parameters. On the contrary, reduction in kernel hardness did not significantly affect global quality score of spaghetti, whereas SDL showed a small, but significant, increase of the bread loaf volume. These results suggest that modulation of kernel hardness in durum wheat does not impair its pasta-making potential and may improve its baking performance. Availability on the wheat scenario of soft-textured durum wheat genotypes may have important practical and useful implications for breeding multiple-purpose durum wheat (pasta, bread, biscuits). Crosses are on going to improve the yellow index which are consistently lower in the finer flour and semolina of soft-textured durum wheat.

Keywords: kernel texture, durum wheat breeding, pastamaking quality, breadmaking quality, puroindolines.

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Introduction

Kernel hardness is a main determinant of end product quality because of its strong effects on milling conditions, granularity of flour and starch granule integrity. In particular, common wheat (*Triticum aestivum* L.) cultivars can be divided into three endosperm-texture classes based on their average SKCS (Single Kernel Characterization System) values, i.e. soft, medium hard and hard. On the contrary, all durum wheat (*T. turgidum* ssp *durum*) cultivars are characterized by an extra-hard kernel texture with SKCS index >90. This extremely hard texture is mainly due to the absence of PIN-A and PIN-B, two basic, tryptophan- and cysteine-rich polypeptides encoded by two closely linked genes named *Pina-D1* and *Pinb-D1*, located in the distal part of the short arm of chromosome 5D (Mattern *et al.*, 1973; Gautier *et al.*, 1994) and consequently absent in AB-genome durum wheat. Extra-hard durum wheat grain is mainly ground to make semolina for the production of pasta and cous-cous and, in Mediterranean countries, it is also used for breads of all types (Quaglia, 1988). 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 and Pogna, 1989; Peña *et al.*, 1994; Boggini *et al.*, 1995; Liu *et al.*, 1996). 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). In order to insert puroindoline genes into durum wheat, 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*. The resulting soft-textured plants devoid of chromosome 5D were used as the male parent in crosses with commercial durum wheat cv. Colosseo (Gazza *et al.*, 2008) and three F_6 plants emizygous at the *Pina-D1*/*Pinb-D1* locus from these crosses were self-pollinated for three generations to develop six F_9 lines, i.e. three Soft Durum Lines (SDL) homozygous for wild-type alleles *Pina-D1a* and *Pinb-D1a*, and three Hard Durum Lines (HDL), lacking the *Pina-D1* and *Pinb-D1* genes.

These soft-textured and hard-textured durum wheat lines are compared for their milling properties, rheological characteristics, pastamaking and breadmaking quality. In addition, in order to reduce plant height a selected SDL line was crossed with durum wheat cv. Simeto and 17 F_6 progeny lines were evaluated in terms of stability for their short height, soft texture and gluten quality.

Materials and methods

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).

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

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 (3Kg) from soft-textured and hard-textured lines were milled with (i) the MCK Buhler experimental mill for durum wheat, (ii) the MLU 202 Buhler experimental mill for common wheat or (iii) the Bona 4RB (Bona, Italy) experimental mill for common wheat. Color of semolina and raw pasta was measured by a Tristimulus colorimeter (Chroma Meter CR-400; Minolta, Milan, Italy), using the CIELab colour space coordinates L* (lightness), a* (red-green chromaticity), and b* (yellow-blue chromaticity), and the D65 illuminant. The milled samples were analyzed with the Chopin Alveograph (Chopin, Villeneuve La Garenne, France) according to the manufacturer's instructions as modified by D'Egidio *et al.* (1990). In addition, the flour samples obtained with the MLU 202 Buhler experimental mill were analyzed with the Brabender (South Hackensack, NJ) farinograph. Flour and semolina obtained from each soft-textured or hard-textured line with the MCK Buhler experimental mill for durum wheat were combined 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 (1.65 mm in diameter) using a laboratory press. After drying at 50°C for 20 h, spaghetti (100 g) were cooked and evaluated for firmness, stickiness and bulkiness by a trained panel of three experts as described by D'Egidio *et al.* (1990).

Bread was baked according to the AACC Method 10-10B with minor modifications (Cattaneo and Borghi, 1979), using flour samples obtained with the milling for common wheat. Loaf volume was determined by rapeseed displacement.

All data are the means of at least duplicate determinations. Data were statistically evaluated by Student's *t* test or analysis of variance.

Results and Discussion

PCR amplifications of genomic DNA with primer pairs specific for seven microsatellites located on 5D chromosome suggested that SDLs contain only a small 5DS fragment, inferior to 14.4 cM in size. Upon A-PAGE fractionation, soft textured durum wheat lines were found to accumulate PIN-A and PIN-B on the surface of their starch granules in amounts comparable to those observed in soft-textured common wheat cultivars. Accumulation of puroindolines reduced SDLs mean SKCS indexes to 19.9 - 23.6, which are typical of soft-textured common wheat cultivars whereas hard-textured durum wheat lines HDL were similar to durum wheat varieties in lacking both puroindolines. According to SDS-PAGE fractionation, all durum wheat lines produced in the present study exhibited LMW-2 glutenin subunits, which are associated with superior gluten strength (Pogna *et al.*, 1990), and inherited HMW glutenin subunit pair 6+8 from Langdon 5D(5B) substitution line.

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 was approximately 24% higher than that of HDLs, and even greater (about 60%) after milling with the MCK Buhler mill for durum wheat. Grain softness strongly decreased farinograph water absorption and consequently resulted in inferior dough tenacity (P), strength (W) and P/L ratio of SDLs with respect to HDLs. Moreover, the lower starch damage accounts for the higher farinograph dough stability and mixing tolerance of SDL milling products, which likely derives from their lower water absorption. It is noteworthy that the substantial variation in water absorption and rheological properties associated with the contrasting kernel textures of the durum wheat lines did not significantly affect firmness, stickiness and bulkiness of spaghetti. In addition, HDLs and SDLs did not differ significantly for their pasta-making quality as determined by the global quality score, and were comparable with high-quality durum wheat cultivars grown in Italy. On the other hand, soft-textured lines showed a small, but significant, increase of the bread loaf volume (approximately 10%) compared with their hard-textured counterparts. These results suggest that modulation of kernel hardness in durum wheat does not impair its pasta-making potential, and may improve its baking performance.

As compared with HDLs, the soft texture of SDLs resulted in significant lower yellow and brown indexes in both flour and semolina fractions obtained with the MCK Buhler mill. This suggest that color was strongly related to the particle size of the milling fractions, yellowness b* and brownness (100 - L*) being consistently and significantly lower in the finer flour and semolina of SDLs.

The high plant height (>120 cm) of SDLs and HDLs resulted in partial lodging at harvesting (until 60%). In order to breed shorter cultivars, an SDL line was crossed with durum wheat cv Simeto. Segregation of allele *Pina-D1a* coding for wild-type PIN-A was followed by PCR amplification on single F_2 plants, whereas segregation of texture in F_3 kernels produced by each F_2 plant was determined by SKCS. Amongst the F_6 resulting progeny, ten soft-textured individuals, <85 cm in height, were selected (Table 1). Moreover, three genotypes (12-81, 12-88 and 12-91) were found to contain HMW glutenin subunit pair 7+8 (Fig. 1), which are associated to a superior gluten strength. Line 12-91 was selected for its good score in terms of reduced plant height, high TKW and soft texture of the kernel and multiplicated over two years to obtain enough seeds to perform a milling test at industrial scale.

Genotype	Plant height ± SD (cm)	SKCS ± SD	Seed weight±SD (mg)
12.76	98.0±13.3	13.6±13.7	58.7±11.2
12.77	96.1±11.2	14.5±13.4	61.7±8.4
12.78	81.1±5.6	0.2±13.6	58.8±10.5
12.79	78.7±7.6	0.1±15.5	57.8±8.9
12.80	77.1±6.4	4.6±15.4	59.7±10.7
12.81	86.0±6.3	10.7±15.2	51.2±12.2
12.82	78.3±6.9	2.6±15.1	60.5±10.0
12.83	78.9±5.1	-2.1±14.4	58.8±11.2
12.84	102.7±6.1	6.6±12.3	56.0±9.1
12.85	75.5±9.2	-9.2±14.0	59.3±8.9
12.86	73.9±5.3	-10.1±12.9	62.1±9.45
12.87	74.8±6.2	39.2±30.4	49.8±12.2
12.88	74.5±5.3	35.4±34.0	58.5±9.3
12.89	95.5±7.7	3.3±13.0	47.9±10.2
12.90	92.2±6.1	2.0±13.8	51.6±8.9
12.91	**68.5±4.5**	**6.5±14.5**	**55.4±12.3**
12.92	70.7±5.0	-5.3±15.3	59.7±12.5

Table 1. Mean values of plant height, SKCS indexes and seed weight of 17F_6 individuals of the cross between SDL1 and durum wheat cv Simeto.

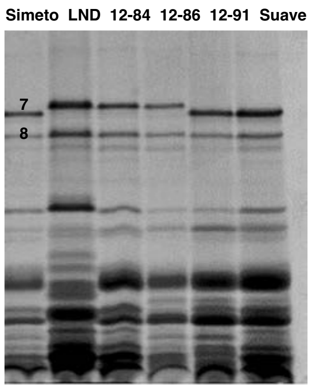

Fig. 1. SDS-PAGE fractionation of storage proteins of lines obtained from the cross SDL x durum wheat cv Simeto. HMW glutenin subunits pair 7+8 is indicated. LND: durum wheat cv Langdon.

In addition this line has been proposed for the commercial development of a durum wheat soft cultivar with the name of Suave in collaboration with the Italian seed company ISEA Srl. In the two years of testing, the hardness indexes of the SDL line varied from 0 to 5.5 as a super-soft hexaploid common wheat. When samples were milled with Bhuler Mill for durum wheat, the soft durum showed a flour yield of 38.9% compared to that of control durum wheat of 25.8%, whereas using a mill for common wheat the SDL had a flour yield of 31.5% compared to that of a control common wheat of 41.7%. On average uptake of water of control durum wheat 68.6% was about 17% higher than that of SDL granulars (58.6%) and about 25% higher than that of control common wheat (54.7%), likely reflecting the high starch damage of the milling products from hard kernels compared with soft ones. Indeed, damaged starch resulted to be up to 23.4% in durum wheat, 13.9% in common wheat and of 10.9% in soft durum. Alveograph W values were higher in durum (265 $Jx10^{-4}$) and common wheat (254 $Jx10^{-4}$) compared to soft durum (173 $Jx10^{-4}$); noteworthy durum wheat have low alveograph L values, an indicator of dough extensibility, which resulted in high P/L ratio (1.43) compared with soft durum wheat (0.51). This ratio is a measurement of the balance between elasticity (P) and extensibility (L), suggesting that soft durum wheat could show a better breadmaking attitude than the extra hard textured durum wheat. Finally, as compared with durum wheat, soft durum resulted in lower yellow color index (b*18.98 *vs* 10.01, respectively); this is a clear indicator that color was strongly related to the particle size of the milling fractions being consistently lower for the finer SDL flours than durum wheat semolina. However, since yellowness is an important trait that affects the color of end-use products and the consumer's choice, breeding activity is on going by crossing soft durum line Suave with high yellow colour varieties.

Conclusions

Durum wheat lines homozygous for a <14.4 cM terminal fragment of chromosome 5D containing the *Pina-D1a/Pinb-D1a* alleles showed SKCS values typical of soft-textured kernels. Softening effect resulted in a about 24% higher flour extraction rates compared with hard-textured lines. Spaghetti cooking quality was unaffected by kernel hardness, whereas loaf volume exhibited a 10% increase associate with kernel softening. Availability of soft-textured durum wheat genotypes may have important practical and useful implications for breeding multiple-purpose durum wheat (pasta, bread, biscuits and other oven products), and for technological operations of industrial interest, which are on going.

Acknowledgements

Authors thank Pierino Cacciatori of CREA-IT for his super visioning of the field trials and D.ssa Daria Scarano of ISEA SrL for her breeding activity.

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